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# *The Journal* *of* *Laboratory and Clinical* *Medicine*

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# *The Journal of Laboratory and Clinical Medicine*

VOL. II.

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No. 1.

## ORIGINAL ARTICLES

### URIC ACID IN ITS RELATIONS TO METABOLISM\*

BY STANLEY R. BENEDICT, PH.D., NEW YORK CITY.

ONE hundred and forty years ago Scheele<sup>1</sup> first isolated uric acid from urine and from urinary calculi. He showed that the substance was acid in properties by dissolving it in lime-water and reprecipitating it with acid. Twelve years later Pearson<sup>2</sup> found uric acid in the tophi of gout, thus laying the foundation of Garrod's theory of this disease which was published half a century later.

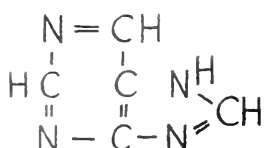
Scheele and Fourcroy<sup>3</sup> and others studied the chemical properties of uric acid in a rudimentary way, but the work of Liebig and Wöhler,<sup>4</sup> published in 1838 gives the first account of carefully conducted experiments in this field. These investigators isolated and studied practically all of the oxidation products of uric acid which are known to this day. Perhaps the most important portion of their work from the biological standpoint was the demonstration that allantoin, which had been previously isolated from the amniotic fluid of the cow, could be obtained by the oxidation of uric acid. Subsequently Moritz<sup>5</sup> showed that suitable oxidizing agents may convert uric acid into oxalic acid and urea, both well known biological products.

Among the numerous papers dealing with the chemistry of uric acid, that of Medicus,<sup>6</sup> published in 1875, is of peculiar interest. In this paper Medicus proposed for uric acid the formula which seven years later Emil Fisher showed to be correct—a beautiful example of a chemical prophecy, if we may use that term.

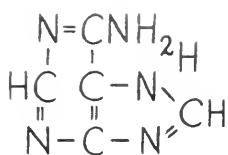
Uric acid is the most highly oxidized member of a group of compounds termed "purines," the most important representatives of which, adenin, guanin, hypoxanthin, xanthin, theobromin, caffein, and uric acid, are represented in Table I.

\*Lecture delivered before the Harvey Society, New York, April 8, 1916.  
From the Department of Chemistry, Cornell University Medical College.



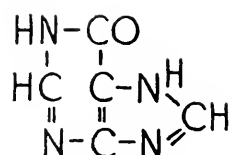


Purin



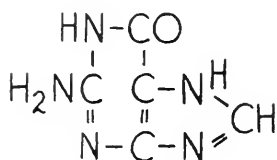
Adenin

G=aminopurin



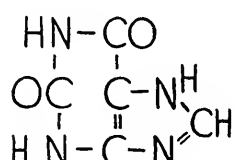
Hypoxanthin

G=oxypurin



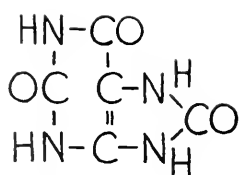
Guanin

2=amino-G=oxypurin



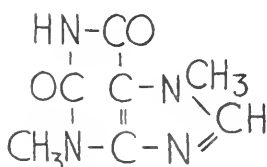
Xanthin

2=G=dioxyurin



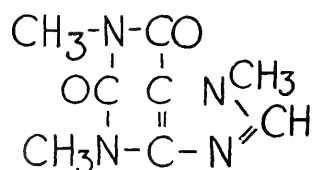
Uric Acid

2=6=8=Trioxypurin



Theobromin

3=7=dimethylxanthin



Caffein

1=3=7=trimethylxanthin

Table 1.

In coming to consider the physiology of uric acid, we may note in passing its remarkably wide distribution in the animal kingdom. It is found in nearly every animal form, from insects to man. Garrod found it in all insects examined except spiders, which excrete guanine in place of uric acid.<sup>7</sup> Hopkins<sup>8</sup> found that the powdery scales on the wings of butterflies consist of uric acid. Birds and reptiles excrete large quantities of the substance, which has also been found in the blood and urine of practically all mammals examined.

We shall now consider the substances from which uric acid may be formed in the animal body and the mechanism of such formation. In the days of Liebig the current view of the origin of uric acid was that it represented a partial oxidation product of all protein materials. If the body had a rich oxygen supply it was believed that nearly all the uric acid formed was further oxidized, while if the oxygen supply was deficient, uric acid was destroyed in much smaller proportion, and gout or similar conditions were likely to develop. This view was accepted for a long time and it was not until the early eighties that Kossel<sup>9</sup> laid the foundation stones of our present theories of uric acid formation. A great many men have aided in working out the problem, but we must mention especially the names of Horbaczewski, Kruger and Schmidt, Burian, Schittenhelm, Jones, and Spitzer on the biological side, and of von Baeyer, Emil Fisher and P. A. Levene on the chemical side. von Baeyer and Fisher worked out the formulæ of the purines and Levene solved the question of the structure of the nucleic acids.

Kossel's work showed that certain purines were to be found among the decomposition products of the widely distributed nucleoproteins. Fisher had already shown the close chemical relationship of these purine bodies to uric acid and Kossel at once suggested that they might constitute the source of uric acid in the animal body. A number of experimenters, on account of unsuitable technic, both as to analytical methods and animals employed, failed to find experimental proof for Kossel's suggestion. Finally, however, Horbaczewski,<sup>10</sup> in a series of beautiful experiments, gave the first demonstration that animal tissues can transform the purines of nuclear material into uric acid. By digesting a tissue rich in nucleic acid, such as the spleen, with blood, he obtained the purine bases hypoxanthin and xanthin in the absence of oxygen, or uric acid when oxygen was present. Horbaczewski also found that feeding of spleen or other glands to either man or the rabbit was followed by an increase in the uric acid eliminated in the urine.

Following Horbaczewski's work, studies were undertaken by various investigators to find the mechanism by which uric acid is formed from nucleic acids in the body. In this field the researches of Schittenhelm and especially of Jones stand out preeminently.

Levene has shown that nucleic acids represent a grouping together of several simple so-called nucleotides. A typical example of a simple nucleotide is guanylic acid (Table 2) in which guamin is linked to phosphoric acid by means of a pentose radicle.

It is from the purine portion of the nucleic acids that uric acid has its chief origin in mammals. The process of the transformation is somewhat complex. The work of Schittenhelm and of Jones has shown that there are at least nine

separate enzymes which may be concerned in it. We may briefly indicate the paths for the transformation of the purines of nucleic acid into uric acid as follows: The initial splitting may be into mono-nucleotides, such as guanylic acid. An enzyme capable of effecting this splitting probably occurs in yeast and has been found in the pancreas of some species. A second type of nuclease, termed "phospho-nuclease," instead of splitting the molecule into mono-nucleotides, will split off phosphoric acid either from mono- or poly-nucleotides, leaving the purine base joined to the carbohydrate as in guanosin. A third type of enzyme splits off purine bases from the original molecule, leaving the phosphoric acid and carbohydrate radicles joined together.

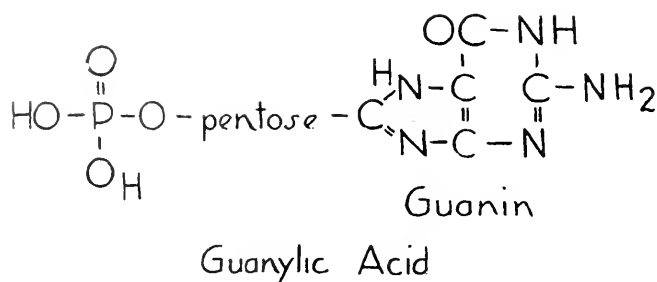


Table 2.

So far as the purine portion of the molecule is concerned, the initial splitting by nuclease may therefore give rise to either free purine (as guanine) or to purine joined to carbohydrate, as in guanosin. The guanine is converted into uric acid through two steps. First the enzyme guanine splits off  $\text{NH}_2$  and replaces it by an oxygen atom giving xanthine. This in turn is acted upon by xanthine oxidase which converts it into uric acid through the addition of an atom of oxygen. The transformation of the purine in guanosin may take place in either of two ways. The base may be set free by a hydrolytic enzyme and be then converted into uric acid, as we have just indicated, or the  $\text{NH}_2$  may be replaced while the purine is still attached to carbohydrate, giving rise to xanthosine, which is then split into its two components. The xanthine is then directly oxidized to uric acid. Where the original purine is adenine instead of guanine the steps involved will be exactly analogous, with the additional step of oxidation of the hypoxanthine formed by deamidation of the adenine to xanthine.

The enzymes concerned in these transformations show widely different distribution for different species. For the most part they are found in the liver, spleen, pancreas and thymus. It is worthy of note that adenase, the enzyme which transforms adenine into hypoxanthine, cannot be demonstrated for human tissues *in vitro*. Guanine is lacking in the pig, which is subject to guanine gout.<sup>21</sup>

In mammals the preformed purines of nucleic acid constitute the chief, if not the only source of uric acid. In birds and reptiles this is not the case. Between 60 and 70 per cent of the total nitrogen of the urine in these species is eliminated in the form of uric acid, and it is obvious that, in such animals, there must be sources of uric acid other than the nucleic acids. Minkowski<sup>12</sup> has

made a special study of this problem in geese and has shown that in this species uric acid can be synthesized from the very simple compound ammonium sarcosylactate. In geese with extirpated livers uric acid almost wholly disappears from the urine, its place being taken by ammonia and sarcosyl-lactic acid. After Minkowski's work Kowalewski and Salaskin<sup>13</sup> furnished final corroboration of his results by showing that if blood containing ammonium lactate be perfused through an isolated goose liver it is changed into uric acid. The nitrogen of urea and of various amino acids has also been shown to be converted into uric acid under such conditions.

There is thus a two-fold source of uric acid in birds; viz., from the nucleic acids and from purine-free nitrogenous compounds. The question naturally arises as to whether there may be such a two-fold origin of uric acid in mammals.

For the young animal this question is easily answered in the affirmative. A growing animal on a milk diet, for instance, receives no nuclein or other purine-containing substance in the food, and yet eliminates uric acid and other purines in the urine and synthesizes a large amount of nuclear material for the body cells. Hence, such an animal must have the power of synthesis of purines from nonpurine materials. In the case of adult animals the question is not so readily answered. The food here constantly contains appreciable quantities of purines, and the impression has prevailed very widely that these food-purines constitute the sole source of the purine bodies catabolized in the adult mammal. At least, definite proof has been lacking that this is not the case, since the ingestion of nonpurine food substances of widely varying structure has not led to any specific increase in the uric acid eliminated. We shall return to this question later. In the meantime we may consider some of the factors which appear to influence uric acid formation, especially in man.

Burian and Schur<sup>14</sup> were the first to call attention to the fact that under ordinary conditions of diet there must be a double source of uric acid in the adult mammal; viz., the nuclear or purine material ingested in the food, and the nuclear material catabolized by the body from its own tissues. To the uric acid formed from the food purines Burian and Schur gave the name "exogenous" uric acid, indicating its outside origin, while the uric acid formed from the body's own store of nuclear material was termed "endogenous" uric acid. In accordance with this view it is found that when a man changes from an ordinary mixed diet to one which contains no nuclear or purine material the uric acid eliminated falls markedly in amount but does not by any means wholly disappear. That portion which does disappear is obviously exogenous in origin; that which persists is endogenous.

Studies on exogenous uric acid formation have been carried out by a number of observers. Burian and Schur, Minkowski, Kruger and Schmid, and more recently Mendel and Lyman, in an interesting and extensive investigation, have reported valuable observations upon the conversion of free purine bases into uric acid in man. Mendel and Lyman<sup>15</sup> found that about 60 per cent of ingested hypoxanthin, 50 per cent of xanthin, 19-30 per cent of guanine and 30-37 per cent of adenine were eliminated as uric acid.

Although uric acid elimination may be much increased after the ingestion

of glandular tissues, such as thymus, only a small fraction of the purine nitrogen reappears in the urine as uric acid. Givens and Hunter<sup>16</sup> have recently reported similar results following ingestion of pure sodium nucleate. What becomes of the balance of the purine nitrogen in these experiments is unknown. The suggestion that it undergoes bacterial decomposition in the intestinal tract is not very satisfactory, in view of the very high percentage which is usually lost.

We may summarize the findings concerning exogenous uric acid by saying that it seems definitely proven that purines, ingested either as free base or in the form of nucleic acid, are at least partially converted into uric acid. The possibility that the purines may have some other path of catabolism than through uric acid has not been excluded.

In a discussion of endogenous uric acid I am reminded of a statement made by Professor Mendel in his lecture before the Harvey Society ten years ago upon the subject of uric acid formation. In summarizing some problems for the future Professor Mendel said, "We assuredly need to know more about the origin and significance of endogenous uric acid." It is, perhaps, a sad commentary upon progress that after these ten years it seems necessary to alter Professor Mendel's statement and say, "We want assuredly to know *something* about the origin and significance of endogenous uric acid." The subject is almost chaos and we have neither time nor inclination to enter into a discussion of the conflicting results of various investigators. There are, however, a few facts which may bear scrutiny. The lowest possible level of uric acid excretion seems to be reached upon a diet very poor in nitrogen but which yields calories enough to protect most of the body's own tissues. Folin called attention to this fact some ten years ago and it has recently been corroborated in an interesting paper by Ringer and collaborators. If the nitrogen intake be slightly higher than the minimum level obtained with a starch and cream diet, there is a slightly greater output of uric acid. If the protein intake be again raised there is no increase in the uric acid output, at least until the nitrogen ingested is well above that contained in our ordinary diets. With a protein intake of 30 or 40 grams of nitrogen per day the uric acid elimination will again show a marked rise. This latter point is shown very beautifully in a recent paper by Taylor and Rose.<sup>17</sup> It is probable that the extra uric acid eliminated in such an experiment is not formed from the protein ingested but rather represents body nuclear material broken down under the influence of the forced protein feeding, a possibility suggested by Taylor and others, or perhaps the excessive activity of the digestive glands may be an important factor in such cases.

Concerning the organs or tissues which may be directly responsible for the production of uric acid, very little can be said. There are only two theories which deserve mention in this connection. One of these, suggested by Burian, assumes that the free hypoxanthin of muscle tissue is converted into uric acid and that such conversion is increased by muscular work. Burian's experiments seem to support this view, but Siven<sup>18</sup> was not able to reach definite conclusions as a result of his work in this connection. This latter investigator found that uric acid elimination was higher during the waking hours than during the night but was unable to demonstrate an effect of muscular work. In spite of a recent paper by Ringer<sup>19</sup> and his coworkers which contains a single

experiment supporting Burian's view, we must conclude that at present nothing can be definitely stated concerning a possible influence of muscular work upon uric acid elimination.

The view that glandular activity, especially that of the digestive glands, is responsible for the formation of some or all of the endogenous uric acid, was proposed many years ago by Mares and has been the subject of much work and more controversy. Siven has opposed the theory, while Hopkins and Hope,<sup>20</sup> Smetanka<sup>21</sup> and others have offered evidence in its favor. Mendel and Stehl<sup>22</sup> recently reported further experiments which favor the view that the digestive glands may contribute to uric acid formation. Their results, like those of Smetanka, seem to show a definite rise in uric acid elimination following an ingestion of purine-free food (protein or fat plus carbohydrate). The hourly variations in uric acid elimination during fasting are so wide in the work reported by Mendel and Stehl that one must be cautious in drawing conclusions from their experiments. The Folin-Shaffer method for uric acid determinations which they used is hardly suitable for determining *hourly* uric acid elimination. A repetition of their work employing the micro method of analysis for uric acid is urgently called for. Nevertheless, we may say tentatively that the balance of evidence is in favor of the view that the digestive glands may contribute to uric acid formation.

The urine of practically all mammals except man and the anthropoid ape contains an oxidation product of uric acid, allantoin, in considerable quantity and the uric acid is correspondingly diminished, or almost absent. Furthermore, various tissues of these species which eliminate large amounts of allantoin in the urine have been found to have the power of destroying uric acid *in vitro* with a corresponding formation of allantoin. The tissues of man and the higher apes do not exhibit this power of uric acid destruction and only minimal traces of allantoin occur in the urine of these species. Hence, it is concluded that uric acid is certainly not the chief end product of purine metabolism in any mammal so far studied except man and his first cousin. By determining the ratio of allantoin nitrogen to uric acid nitrogen plus allantoin nitrogen in the urine, Hunter and Givens have calculated what they call the "uricolytic index," or power of uric acid destruction for various species. This index shows uric acid destruction varying from 79 to 98 per cent in widely separated species of the lower mammals, while in man and the chimpanzee the index would be approximately zero.

Schittenhelm has long contended that man possesses quite a marked power of uric acid destruction in spite of the fact that Wiechowski has been able to recover in the urine nearly all of the uric acid injected subcutaneously into man and in spite of the further fact that no power of destruction of uric acid can be demonstrated in human tissues postmortem. While the evidence which Schittenhelm offers in favor of uric acid destruction by man is far from convincing and has been recently partly refuted in a paper by Fine,<sup>23</sup> it is also true that the evidence against this view is not final. It has been found, as will be mentioned later, that certain free purine bases behave differently in the body from the same purines when given in combination in nucleic acid, and if it should be shown that uric acid or the amino purines may go to other products

than allantoin, then Schittenhelm's view may have a certain degree of plausibility. If, for instance, uric acid, as formed in the organism were in a combination, it is possible that such uric acid might be destroyed, although free uric acid, either injected subcutaneously or mixed with tissue post mortem, might escape such destruction. As we shall see later, there are some facts which might lend support to these suggestions.

The study of uric acid metabolism has been much hampered by the fact that no animal has been available for experimental purposes in which uric acid is an end product of metabolism or in which the essential features of human purine metabolism appear to be duplicated. It is therefore of interest to know that recently an animal has been found which appears to fulfill these conditions and in which it seems probable that we shall find some of the missing links between the purine metabolism of man and other mammals.

The animal in question is the Dalmatian breed of dog. This breed is commonly known as the coach or carriage dog and is characterized by its spotted or mottled appearance. A specimen of this breed of dog encountered in the laboratory a little over a year ago showed a very peculiar anomaly of uric acid metabolism. Simple addition of hydrochloric acid to the urine of this animal was followed by the immediate formation of a heavy amorphous precipitate. On standing a short time this precipitate assumed the characteristic form of pigmented uric acid crystals. Isolation and analysis of the purified crystals showed them to consist of uric acid. The substance has repeatedly been isolated in large quantities from this urine and analyzed. An examination of the urine of this animal showed that upon a purine-free diet it was eliminating almost as much uric acid per day as would an average sized man on a similar diet, and this in spite of the fact that the dog weighs only about ten kilograms. Furthermore, uric acid injected under the skin was eliminated quantitatively as such in the urine. These findings are the more noteworthy because the ordinary dog shows a destruction of from 98 to 100 per cent of uric acid either subcutaneously introduced or that formed in metabolism. It was at first supposed that the anomaly of purine metabolism existed only in the one individual but further examination has shown that it is probably a peculiarity of the breed of Dalmatians. We have examined five animals more or less pure Dalmatians and in four of them have found a very high uric acid elimination. The one exception was obviously not of very pure breed.

It may be noted in passing that it seems difficult to find any analogy for the perversion of purine metabolism in the Dalmatian. Conditions such as cystinuria or pentosuria, which are found in some human individuals, would be closest to it, but in these cases the peculiarity is individual and not racial.

In connection with the experiments reported upon the Dalmatian dog, I desire to express my indebtedness to Mr. Emil Osterberg of our laboratory, whose constant and most efficient help has been invaluable.

Table No. 3 shows the general metabolism of our Dalmatian as represented by the urinary findings upon purine-free diets with varying nitrogen content. It will be noted that the uric acid output is not increased following a four-fold increase in the nitrogen content of the food. The allantoin (determined



by Wiekowski's method) shows, on the contrary, a distinct rise following the increased nitrogen ingestion.

TABLE No. 3.

Total N. grms.	Creatinin N. grms.	Uric Acid N. grms.	Ailantoin N grms.	Remarks
4.8	0.103	0.123		Diet contains 5.9 grms. total N.
5.0	0.106	0.119	0.057	
4.9	0.103	0.126	0.064	
4.9	0.105	0.121	0.061	
5.1	0.104	0.120	0.051	
16.6	0.106	0.117	0.037	Diet contains 24.07 grms. total N.
18.4	0.110	0.119	0.107	
19.8	0.109	0.128	0.103	
17.5	0.111	0.123	0.102	
15.2	0.104	0.116	0.083	
5.4	0.104	0.154	0.073	Diet contains 2.03 grms. total N.
4.1	0.100	0.139		
3.4	0.100	0.139	0.068	
3.0	0.094	0.141	0.054	
2.8	0.091	0.139	0.059	
2.7	0.084	0.140	0.057	

For a period of nearly a year the animal has been upon a purine-free diet and during nearly all this time the uric acid elimination has been determined daily. As a result of this study we can definitely conclude that the adult mammal can synthesize purine from nonpurine material. During the period of observation the dog maintained a constant body weight and eliminated a total of more than 100 grams of uric acid. Not 10 per cent of this quantity of uric acid could have come from the preformed purine of the animal's tissues. So far as I am aware, this experiment is the first which definitely shows that an adult mammal can synthesize the purine nucleus.

The influence of caffen ingestion upon uric acid elimination is a question which has received much study. A number of investigators have failed to note any increase in uric acid output after the feeding of caffen to man.<sup>24</sup> Taylor<sup>25</sup> obtained the opposite result but the general conclusion has been that caffen ingestion in man does not lead to increased uric acid formation.

Table No. 4 records two experiments upon our Dalmatian to determine the effect of caffen given subcutaneously. A daily dose of 100 milligrams of the drug is followed by a slight decrease in the uric acid output. Although the variation is not marked, we have obtained it in each of three similar experiments. With a larger dose (200 milligrams daily) of caffen there is scarcely any perceptible effect upon the uric acid output but there is a very notable retention of nitrogen during this period.

In Table No. 5 is recorded an experiment to study the effect of caffen ingestion upon the uric acid output in man. The micro method of analysis was employed for the uric acid determination, a method which we have found to be highly accurate. The subject was placed upon a purine-free diet which was kept approximately constant but which was not weighed. During the prelim-

inary period five cups of a caffein-free coffee (Kaffee Hag) were ingested daily. During the caffein period the diet was just the same but to each of the five cups of coffee taken were added 200 milligrams of caffein, making a total of 1 gram of caffein per day. The uric acid figures of the urine showed a slight, but

TABLE No. 4.

Total N. grms.	Uric acid N. grms.	Allantoin N. grms.	Remarks.
5.37	0.129	0.213	
5.65	0.126		
6.07	0.128		
5.52	0.125	0.192	100 mgm. caffein.
5.41	0.113		" " "
5.23	0.116		" " "
5.13	0.115		
5.20	0.128		
5.34	0.127		
5.31	0.128		
4.82	0.136		200 mgm. caffein.
4.80	0.139		" " "
4.60	0.127		" " "
4.22	0.123		" " "
4.22	0.128		" " "
4.51	0.125		
4.72	0.124		
4.90	0.122		
4.96	0.127		

definite and progressive increase during the caffein period, which increase was still somewhat apparent for two days after the caffein intake was stopped. This experiment was so carefully conducted and the results were so clear cut, that I believe we are justified in concluding from it that caffein may lead to increased

TABLE No. 5.

SUBJECT E. O.

Volume C.C.	Total N grms.	Creatinin N grms.	Uric acid grms.	Remarks.
1300	12.60	0.495	0.540	
1290	13.12	0.486	0.522	
1300	13.74	0.508	0.540	
1240	10.76	0.504	<b>0.558</b>	1 grm. caffein.
1640	10.42	0.484	<b>0.564</b>	" " "
1210	8.03	0.519	<b>0.642</b>	" " "
1080	10.74	0.531	<b>0.702</b>	" " "
1200	9.05	0.543	0.636	
1100	13.53	0.531	0.600	
1400	12.51	0.502	0.546	

uric acid formation in man, and furthermore, as evidenced in Tables Nos. 3 and 4, that it may lead to some nitrogen retention. Indeed, on the basis of our experiments, we may question whether caffein, even in small doses, is quite so innocuous a substance as has been assumed.

In Table No. 6 experiments are reported showing the effect of the addition of nuclear material in the form of thymus gland to the diet of the Dalmatian. It will be noted that after the thymus ingestion the increase in the uric acid eliminated is not nearly so great as the increase of allantoin. Thus, on the purine-free diet the uric acid nitrogen is more than double that of the allantoin, while after the thymus ingestion the increase in uric acid nitrogen eliminated is only about one-half the increase to be found in the allantoin nitrogen. These results might be taken to indicate that exogenous nuclear material undergoes catabolism along different lines from that of the endogenous purine-containing material. The question involved will require further work before any definite conclusion can be drawn. This is especially evident when we note (as shown in Table No. 6) that the uric acid administered subcutaneously is followed by a marked increase in the allantoin output, and this in spite of the fact that the uric acid is recovered quantitatively as such in the urine. It seems probable that uric acid and allantoin are interrelated in metabolism in other ways than have been heretofore assumed.

TABLE No. 6.

Total N. grms.	Urea + Ammonia N. grms.	Uric Acid N. grms.	Allantoin N. grms.	Increase in Uric Acid N. grms.	Increase in Allantoin N. grms.	Remarks.
4.37	3.86	0.120	0.050			
4.54	4.03	0.125	0.050			Normal diet.
4.82	4.36	0.127	0.050			
6.48	5.87	0.177	0.210	0.223	0.480	Same diet + 100 grms. thymus daily = 0.32 grms. purine N.
6.86	6.15	0.197	0.210			
7.30	6.58	0.200	0.210			
5.75	5.27	0.129	0.058			
5.54	5.08	0.132	0.058			Normal diet.
5.27	4.84	0.137	0.058			
5.18	4.50	0.290	0.115			Same diet + 500 mgm. of uric acid sub- cutaneously.
5.13	4.47	0.299	0.142			
5.00	4.30	0.305	0.133			
5.02	4.54	0.124	0.066			

We finally turn to what may be regarded as essentially the most modern field of uric acid research. I refer here to the investigations of uric acid in blood. This field of work will be irrevocably associated with the name of Otto Folin. For many years Folin, more than any other man either here or abroad, has been a maker of high grade tools for the biochemist. In methods of uric acid research he has recorded another notable achievement. Before the Folin method was available it was questionable whether uric acid existed at all in normal human blood and quantities up to 300 cubic centimeters were necessary for even approximately accurate results with bloods known to contain an excess of uric acid. Now we are able to determine uric acid with accuracy in twenty or even in ten cubic centimeters of normal human blood. The method is equally applicable to the blood of other species.

The finding of uric acid in pathological human blood dates back to the work

of Garrod in 1848. With a truly outrageously inadequate method of analysis this brilliant worker was able to obtain results of permanent value and to demonstrate for the first time that there is an accumulation of uric acid in the blood of gout and nephritis. But from the time of Garrod until the introduction of the Folin method almost no progress was made or even attempted in this field of work.

The recent researches on uric acid in blood have yielded some results of interest in connection with lower animals as well as in man. In order to appreciate these results we must look backward for a moment. A hypothesis to explain gout, based upon the presence of two forms of uric acid in blood, was put forth a number of years ago and the questions involved here have received a great deal of attention from the experimental side ever since. Pfeiffer<sup>26</sup> thought that he had obtained proof of two forms of uric acid in urine but his work was shown to be erroneous. Kossel and Neumann, Goto,<sup>27</sup> and Minkowski called attention to the fact that uric acid seems to form a compound with thymic acid and Minkowski suggested that uric acid may circulate in the body in this form. There has been no experimental proof that this is the case. One interesting observation of Minkowski's has been interpreted as showing that some purines, at least, are catabolized in the body in combination and not as the free base. Minkowski found that when free guanin was given to dogs the gastrointestinal tract showed marked inflammation and a crystalline deposit of what he thought was adenin was to be found in the kidneys. These effects did not follow the ingestion of adenin in the form of nucleic acid. Subsequently, Nicolaier<sup>28</sup> showed that the deposits found in the kidneys after adenin ingestion consisted of di-oxy-adenin, thus showing that the organism may oxidize a purine before splitting off the  $\text{NH}_2$  group. Such observations as these, and especially the more recent work of Bloch,<sup>29</sup> have tended to show that uric acid may exist in the organism in more than one form, but positive evidence has been lacking. Through use of the recent methods of blood analysis it has been shown beyond a doubt that in some mammals, at least, uric acid exists in the blood chiefly in combination. In fresh ox blood, for instance, using the Folin method of determination, we find about one-half of one milligram of uric acid in 100 grams of blood. If, however, the blood filtrate after removal of protein is boiled with hydrochloric acid and then the uric acid determined, it is found that the quantity present is more than 1000 per cent of the figure originally obtained. The same figure is ultimately reached if the whole blood is simply allowed to stand, thus indicating that an enzyme is present in blood which can split the uric acid combination. The combined form of uric acid is contained wholly in the corpuscles of the ox blood. In birds, in which uric acid is an end product, the blood contains none of the combined uric acid and the free uric acid present is almost wholly in the serum. It is of interest to note that the blood of the ox, an animal which eliminates almost no uric acid in the urine, contains actually 50 per cent more uric acid than does that of birds. In the ox blood it is combined and in the bird's blood it is free. These results have been received with considerable skepticism in many quarters, but since the uric acid can be readily quantitatively isolated as such, the correctness of the work is not open to question.<sup>30</sup> The results seem to show that it is probably form

rather than quantity of uric acid in blood which is of importance. These findings have been extended to the blood of other species and the results have shown that, with the exception of man, all mammals probably have two forms of uric acid in the blood. In the case of human blood the data so far available are not conclusive. It is quite probable that here, too, uric acid exists in the blood in at least two forms but they are quite unlike the forms present in ox blood. A new technique is being developed to study this question.

The clinical findings in regard to uric acid in human blood are of considerable interest. The field of work here is new and we must be cautious in drawing conclusions. The recent researches of Folin and his pupils in Boston and of Myers and Fine at the Post-Graduate School in New York have shown that normal human blood contains from one to three milligrams of uric acid in 100 grams of blood. In lead poisoning, in gout, and in nephritis, the uric acid content of the blood is usually markedly increased and the determination of uric acid in the blood of suspected gout is of unquestioned value.

In connection with gout the recent researches have shown that the old idea that in this condition the blood becomes "saturated" with uric acid must be abandoned. The solubility of uric acid in blood serum has been shown to be much greater than the concentration of uric acid occurring in the blood of gout. Furthermore, in nephritis the uric acid content of the blood may be quite as high as in gout for long periods of time without any symptom of uric acid deposition occurring. We have therefore to assume that in gout there is not only a kidney deficiency for uric acid elimination but that there is also a direct vicarious excretion of uric acid from the blood stream into certain tissues where it finally reaches the saturation point and deposits in the form of sodium acid urate. The view of Minkowski and others that the uric acid circulates in gout in some different form from that in the normal, finds some support from the results already referred to with the blood of lower mammals. It is my opinion that this view will prove to be the correct one.

In connection with the use of salicylates and of atophan in gout it is of interest to note that Fine and others have shown that the administration of either of these drugs to gouty patients is followed by a prompt drop in the uric acid content of the blood. Frequently this drop may be so great that the uric acid practically wholly disappears from the blood for a time. With continued administration of either drug, however, the uric acid reaccumulates in the blood. Hence it is of no service to give salicylates or atophan continuously. Whether by alternating these two drugs for a period of a week or two with each, the blood could be kept relatively free from uric acid continuously, has not yet been determined.

Table No. 7, for which I am indebted to Professor Myers of the Post-Graduate Hospital, illustrates the findings of Myers and Fine in regard to the early accumulation of uric acid in the blood in nephritis. From this table we should infer that of all the common products of metabolism uric acid is the first to accumulate in the blood when the kidney function is impaired. Whether uric acid in high concentration in the blood is *per se* toxic to the kidney is not yet known. The frequency with which gout develops into nephritis might lend support to this view. Occasionally, apparently normal individuals are encoun-

tered whose blood has a uric acid concentration of over 3 milligrams per 100 grams of blood. If such individuals could be followed for some years we would probably obtain data of value as to the possible etiological importance of uric acid in nephritis.

TABLE No. 7.

URIC ACID, UREA N. AND CREATININ OF BLOOD IN GOUT AND EARLY AND LATE NEPHRITIS.

Diagnosis.	Uric Acid.	Urea N.	Creat- inin.	Systolic Blood Pressure.
	Mgms. to 100 c.c. blood.			
Typical Cases of Gout.	9.5	13	1.1	230
	8.4	12	2.2	164
	7.2	17	2.4	200
	6.8	14	1.7	
Typical Early Interstitial Nephritis.	9.5	25	2.5	185
	8.0	37	2.7	150
	5.0	37	3.0	130
	7.1	16	2.0	
	6.6	24	3.3	185
	6.3	18	2.1	
	8.7	20	3.6	100
	7.0	33	2.6	117
	6.3	31	2.1	
	6.3	23	2.4	150
	8.0	80	4.8	240
	4.9	17	2.9	170
	8.3	72	3.2	238
	5.3	21	1.9	145
	9.5	44	3.5	210
Chronic Diffuse and Chronic Interstitial Nephritis.	2.5	19	1.9	120
	7.7	67	3.1	
	6.7	17	1.6	165
	8.3	39	2.9	
	6.5	24	3.0	200
Typical Fatal Chronic Interstitial Nephritis.	22.4	236	16.7	210
	15.0	240	20.5	225
	14.3	263	22.2	220
	13.0	90	11.1	265
	8.7	144	11.0	225

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## POISONOUS PROTEINS\*

(Continued from page 861, Vol. I.)

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### Part III.—Protein Fever

IT has been known for a long time that the parenteral introduction of proteins in the animal body may be followed by fever. As early as 1883 Roques collected the literature of this subject and reported his own experimental studies. A few years later Gamaleia made a most important contribution to this subject. The title of this paper is significant and reads as follows: "The Destruction of Bacteria in the Febrile Organism." Gamaleia found that fever follows the parenteral introduction of bacterial protein, both pathogenic and non-pathogenic, both living and dead, consequently he concluded that fever is a result not directly of bacterial growth, but of bacterial destruction in the body. Indeed, he observed that attenuated bacteria often induce a higher and more persistent fever than the virulent forms. When a rabbit is inoculated with a virulent anthrax bacillus fever develops but persists only a few hours, and then the temperature falls below the normal and death occurs. On the other hand, when the second vaccine is used on a fresh animal, fever appears and continues for three days. When a highly virulent anthrax bacillus is employed there may be no fever and death follows within six or seven hours. Gamaleia made similar observations in other infections and came to the following conclusion: "Fever is not a result of bacterial growth, but on the contrary is consequent upon a reaction on the part of the body against the bacteria and leads to their destruction." Furthermore he found that nonpathogenic bacteria, living or dead, lead to the development of fever. I think that these experiments, made more than a quarter of a century ago, furnish strong support of my theory that fever is due to the parenteral destruction of proteins. One year later this work was confirmed by Charrin and Ruffer and was shown to hold good for nonbacterial proteins as well. In 1890 Buchner induced the characteristic phenomena of inflammation—calor, rubor, tumor, and dolor—by the subcutaneous injection of

\*The Herter Lectures for 1916 given in the University and Bellevue Medical School, New York.



diverse bacterial proteins. Krehl and Matthes induced fever by the parenteral administration of albumoses and peptones, but did not obtain constant and uniform results, because as we now know they did not recognize the necessity of regulating the size and frequency of the doses. In 1909 my students and I showed that by regulating the amount and frequency of the dosage we could induce any desired form of fever, acute, fatal, intermittent, remittent or continued.

Inasmuch as I have given elsewhere\* the details of this work I will only reproduce the conclusions and make a few general remarks: (1) Large doses of unbroken protein administered intra-abdominally, subcutaneously or intravenously have no effect on temperature, at least do not cause fever. (2) Small doses, especially when repeated, cause fever, the forms of which may be varied at will by changing the size and frequency of the dosage. (3) The effect of protein injections on the temperature is more prompt and marked in sensitized than in fresh animals. (4) The intravenous injection of laked blood corpuscles from either man or the rabbit causes in the latter even in small quantity, either in single or repeated doses, prompt and marked elevation of temperature. (5) Laked corpuscles after removal of the stroma by filtration have a like effect. (6) Protein fever can be continued for weeks by repeated injections, giving a curve which cannot be distinguished from that of typhoid fever. (7) Protein fever is accompanied by increased nitrogen elimination and gradual wasting. (8) Protein fever includes most instances of clinical fever. (9) Animals killed by experimentally induced fever may die at the height of the fever, but as a rule the temperature falls rapidly before death. (10) Fever induced by repeated injections of bacterial proteins and ending in recovery may be followed by immunity. (11) The serum of animals in which protein fever has been induced digests the homologous protein *in vitro*. In view of recent work on antiferment in blood serum this point needs reinvestigation. (12) Fever is one of the results of the parenteral digestion of proteins. (13) There are two kinds of parenteral proteolytic enzymes, one specific and the other nonspecific. (14) The production or activation of the nonspecific ferment is easily and quickly stimulated. (15) The development of the specific ferment requires a longer time. (16) Sensitization and lytic immunity are different manifestations of the same process. (17) Foreign proteins, living or dead, formed or in solution, when introduced into the blood soon diffuse through the tissues and sensitize the cells. Different proteins have predilection places in which they are deposited and where they are, in large part at least, digested, thus giving rise to the characteristic symptoms and lesions of the different diseases. (18) The subnormal temperature which may occur in the course of a fever or at its termination is due to the rapid liberation of the protein poison, which in small doses causes an elevation, and in larger doses a depression of temperature. (19) Fever *per se* must be regarded as a beneficent phenomenon inasmuch as it results from a process inaugurated by the body cells for the purpose of ridding the body of foreign substances. (20) The evident sources of excessive heat production in fever are the following: (a) That arising from the unusual activity of the cells supplying the enzyme; (b) That arising from the cleavage of the foreign

\*Protein Split Products in Relation to Immunity and Disease, Lea & Febiger, 1913.

protein; (c) That arising from the destructive reaction between the split products from the foreign protein and the proteins of the body.

The above are the conclusions which I drew three years ago from experiments which my students and I had carried out and from a study of the literature of the subject. I did not suppose at the time, nor do I hold now, that all these conclusions are exactly right.

The fundamental fact that the parenteral introduction of proteins may induce fever is founded upon so many independent observations, some of them recorded many years ago, that I do not think it incumbent upon me to seek additional support. Friedberger has, in a most exact way, confirmed the statement that large doses of foreign protein do not, while small doses do elevate the temperature. Moreover, he has shown that a small dose is more effective in sensitized than in unsensitized animals.

Thiele and Embleton have confirmed experimentally the proposition that the parenteral introduction of foreign proteins affects the temperature, causing a rise or fall or having no effect according to the size of the dose. They give the following tables:

EGG-WHITE.

Limits of	NORMAL ANIMAL	SENSITIZED
	grams	grams
Temperature fall .....	0.05	0.005
Constant temperature .....	0.02	0.0002 to 0.0001
Temperature rise .....	0.01 to 0.001	0.0001 to 0.000002

TUBERCLE EMULSION.

Limits of	NORMAL ANIMAL	SENSITIZED
	grams	grams
Temperature fall .....	0.005 to 0.002	0.0005
Constant temperature .....	0.002 to 0.001	0.0001
Temperature rise .....	0.001 to 0.00001	0.00001 to 0.000001

Criticism of the statement that foreign proteins find certain predilection tissues in which they accumulate has been made. Iodine accumulates in the thyroid gland. Mercury induces characteristic lesions in the kidneys. Strychnia selects a definite portion of the nervous tissue on which its action is made manifest. The therapeutic effects of the most approved drugs depends upon their predilection for certain tissues. The recent studies of Rosenow indicate that bacterial proteins do not differ from other poisons in this respect. We are accustomed to think of chemotaxis as acting only between morphologically recognizable bodies, but in reality it is a form of chemism and is dependent upon chemical composition and not on histological structure.

The only one of the above given statements formulated some years ago which has met with any experimental negation is my contention that specific proteases are developed by the parenteral introduction of foreign proteins. I am ready to admit that Friedberger's anaphylatoxin comes from the serum. In fact at the same time that I formulated the proposition concerning protein fever I wrote as follows: "It has been suggested: (a) That the agar or kaolin or bacteria absorb the complement from the serum and that this renders it poisonous. (b) That the poison is preformed in the serum, but that its action is neutralized by some other constituent of the serum which is absorbed by the agar

or kaolin. (c) That the absorption of some constituent of the serum by the agar, kaolin or bacteria leads to a disturbance of the equilibrium of the protein constituents of the serum which as a consequence break up with the liberation of the poison. These suggestions assume that the poison comes from the serum and this may be true." On another page I said: "That the anaphylatoxin comes from the blood serum, the one constant factor in all the experiments in its production, is most probable." Now since the probability has become a certainty, we need not conclude that specific proteases never result from the parenteral introduction of proteins. I have shown that all proteins, including those of blood serum, contain a poison and I am not at all surprised on learning that such a poison in the serum is set free in the production of Friedberger's anaphylatoxin and in the development of Abderhalden's pregnancy test, but these have nothing to do with the development of proteases in smallpox or typhoid fever. At least no such connection has been shown.

#### THE PHENOMENA OF INFECTION.

I have elsewhere gone into some detail concerning the views of the nature of infection which I have developed in my studies on the chemistry and toxicology of bacterial and other proteins. Only a living thing can infect. Injection of diphtheria or tetanus toxin may cause all the symptoms and lesions of the respective diseases, but such injections are artificial procedures and the results are intoxications rather than infections. In this paper I shall omit diseases due to toxins. The infecting agent is a virus and in infections there is a contest between the invader and the native. It is a struggle for food, growth, and reproduction. In the bacterial diseases the structure or the equipment of the invader is quite as complicated and as complete as that of the defender. The contest is between bacterial and body cells and the battlefield may involve only a small part or may extend to every part of the animal's body.

What is the difference between pathogenic and nonpathogenic bacteria? In order for a given bacterium to be pathogenic to a given animal it must be possible for the former to feed upon the latter. All living things feed by means of digestive ferments. Continued life and multiplication are impossible under other conditions. First, in order for a given bacterial to infect a given animal the ferments of the former must be able to digest the proteins of the animal. In the second place the invading cells must not be immediately destroyed by the ferments elaborated by the body cells. There must be a supporting relation between the bacterial cell and the medium, and in infection the body constitutes the medium in which the bacteria grow and multiply. The protein groups split from the medium must fit into the molecular structure of the bacterial cell; otherwise they would be of no service to it. Many kinds of cells may live in the same medium, but for each kind the cleavage of the medium must be specific. From this it follows that the agent by which the cleavage products are secured must be supplied by the cell and must be specific to it.

It follows from what has been said that a bacterium placed in a medium in which its ferment is ineffective cannot grow and multiply. A bacterium which cannot grow and multiply in the animal body cannot cause an infection. Its inability to grow and multiply in the animal body may be due to the fact that its ferments cannot digest or properly break up the proteins of the animal body.

This is one of the reasons why the great majority of bacteria are harmless or nonpathogenic. This, however, is not the sole, and probably not the dominant cause of the failure of so many species of bacteria to do harm to the higher animals. What has been said about the production and utilization of ferments by the bacterial cell is equally true of the body cell. In fact, it is true of every living cell. The body cell has its specific ferments, and the bacterial cell being protein substance is liable to be digested by the ferments elaborated by the body cells. In these simple facts lies the fundamental explanation of all forms of bacterial immunity, either natural or acquired. It will be understood that I am here omitting all reference to the elaboration of toxins and antitoxins.

Ferments are intra- and extra-cellular. All are formed within the cell, but some diffuse into the medium while others do not. In some instances at least, cell permeation by the pabulum is essential to the feeding of the cell. In other cases the ferment accumulates on the surface where digestion proceeds. In others the ferment diffuses into the medium more or less widely from the cell which produces it. Many cells produce both intra- and extra-cellular ferments, and these differ in function.

I am not going into detail concerning cellular ferments. Those of the bacterial cells are easily obtained and have been studied quite elaborately. Some digest proteins, such as gelatine, quickly while others are less prompt and others still have no recognizable effect on this protein. They are easily affected by the presence of certain nonprotein substances, especially carbohydrates. The ferments of the body cells are not so easily obtained and are more difficult of study. However, both the intra- and extra-cellular ferments of the polymorphonuclear corpuscles have been studied in some detail and their destructive action on certain bacteria has been demonstrated. The germicidal action of the blood and its serum has been demonstrated on various species of bacteria.

It may be well to point out some differences between intra- and extra-cellular ferments. The latter are comparable to the enzymes of the alimentary canal. Their function is solely a lytic one. They break up complex proteins into simpler bodies, but these without further treatment are not ready to be built into the cellular structure. The extra-cellular ferments are in a general way destructive in action. The intra-cellular ferments are essentially constructive. They shape the rough blocks and fit them into the molecular structure. In the process of infection the intra-cellular ferments of the bacterial cells are most active. The soluble, simple proteins of the fluids of the animal's body are quickly built into the bacterial cell and growth and multiplication result. Body proteins are converted into bacterial proteins. This process proceeds so smoothly that as a rule during the time when its development is most rapid the host is quite unaware of the presence of his undesired guest. Whole molecules of albumins and globulins are taken into the bacteria and built into the more complicated bacterial cell. This is the period of incubation in an infection. The body cells are not prepared to combat the invader during this period. Finally the body cells react and begin the elaboration of ferments which destroy the bacterial proteins. This is quite a different process. Complex, cellular proteins are split into simpler ones and protein poisons are set free.

During the period of incubation of an infectious disease, the infecting organism supplies the ferment, the simple, soluble proteins of the body fluids con-

stitute the substrate, the process is essentially constructive, no poison is set free and there are no recognizable clinical symptoms. During the active progress of an infectious disease, the body cells supply the ferment, the complex, bacterial, cellular proteins constitute the substrate, the process is essentially destructive, the protein poison is set free, the symptoms of disease appear, lesions more or less destructive develop and life is placed in jeopardy.

The experienced clinician will easily understand that in most infectious diseases the steps in the evolution of the processes are not so clearly defined as indicated in the above statements. They are most typical in uncomplicated cases of yellow fever, typhoid and typhus and in smallpox, but even in these there often are complicating factors. In yellow fever an attempt is made to eliminate the poison into the alimentary canal as is evidenced by black vomit. In typhoid the poison in being excreted into the intestine may lead to perforation. In most infections, the bacterial growth and their disruption overlap. In one part of the body the bacteria continue to grow while in other parts they are being destroyed. In pneumonia life may be endangered by the abundance and extent of the exudate, while in the crisis of this disease autolysis probably plays an important role not only in the destruction of the organisms, but in the removal of the exudate. In many infections lesions develop and impair the efficiency of the body cells. Moreover in destructive lesions the dead tissues of the body must be disposed of and this throws an increased burden on the body cells. In some diseases phagocytosis plays an important role. It must be evident that the engulfment of bacteria by phagocytes is a more conservative method of disposing of the invading cells than their extra-cellular destruction, since in the former the body is protected against the poison liberated by bacterial cleavage. Nothing more dangerous to the infected individual could happen than the sudden cleavage of all the bacteria in his body. The poison liberated in this process would overwhelm him at once. This is a probable explanation of the fact, already referred to, that the case mortality in typhus fever is higher among the well nourished than among the less robust. Bacterial cells, as well as body cells, have means of protecting themselves. The tubercle bacillus through limitless generations of parasitism has developed coatings of fats and waxes which protect them against the action of secretions of body cells quite as efficiently as coats of mail protected our ancestors against the weapons of their time. Moreover, bacterial cells may develop increased resistance or become to some extent immune to the action of body cell secretions. Occasionally bacteria persist in the body for long periods after recovery from the disease and when these are transferred to new hosts they show that they have lost nothing in virulence. Frequently, secondary infections develop and decide the fate of the individual. As someone has said the pyogenic micro-organisms frequently play the last act in the great tragedies of life, tuberculosis, cancer, and syphilis.

#### A CHEMICO-BIOLOGIC CONCEPT OF THE PROTEIN MOLECULE.

Under this heading I wish to formulate certain theories which have developed in my mind during the progress of the work which I have outlined in preceding lectures. Some men seem able to work without developing theories and probably this is best, but I have never worked in that way. It is possibly a fault; if it be, I am ready to confess that I have sinned and continue in the

same old way. I hope that some of the statements which I am about to make will stimulate others to investigate and this I deem of more importance than their truth or falsity.

The protein poison about which all my work has centered is a fact. It has been prepared and studied by so many competent men that its wide distribution in proteins from diverse sources cannot be questioned. Its effects on animals have been widely tested and the general conclusions reached are quite as uniform as those which might be formulated about poisons much longer known. Its chemical structure has not been determined with certainty. The best evidence at hand today seems to indicate that it is not a basic body, and therefore not a protein alkaloid, not a leucomain or a ptomain. It contains no phosphorus and no carbohydrate. In the purest form in which it has been obtained it yields a trace of ash of which phosphorus and chlorine are not essential constituents. Whether this mineral matter is an essential part of the poison or not I do not know. Under any condition in which it has been obtained it is decidedly acid in character and yields amino acids on disruption. It seems to be a polypeptid.

Underhill, whose opinion I esteem highly, concludes that the action of the protein poison on animals is similar in kind but more intense than that of proteoses. I dare say that this is quite right and it conforms with my own observations. I suggested in the Shattuch lectures in 1906 that the protein poison is the chemical nucleus, keystone, or archon of larger and more complicated protein molecules.

The chemism of the protein poison is intense and it combines with various inorganic and organic substances to form more complex molecules, still retaining and imparting to these larger molecules its protein characteristics. Combined with phosphate of lime it forms such phosphoglobulins, so called, as casein. Combined with carbohydrate it develops the glycoproteins and in combination with both phosphorus and carbohydrates, the glyco-nucleo-proteins result. In the last mentioned bodies the protein molecule reaches its most complex form and further development is possible only by polymerization and the aggregation of many protein molecules into cells. At what stage in the evolution of the protein molecule metabolism begins I cannot say, but it is quite evident that multiplication does not begin until the most complex structure has been reached. It seems quite evident that from the beginning the process is a synthetical one.

It is possible to conceive of the beginning of life on the earth, as proceeding in this way. In the intense heat of past geological ages when even carbon existed in the gaseous state this element combined with nitrogen forming cyanogen. With this binary compound under proper conditions the synthesis of the simplest amino acid was possible for cyanogen may react with boiling hydroiodic acid with the development of amino acetic acid and from this the other amino acids found in the protein molecule might have been developed. In this view, proteins in their simplest form may have come into existence long before life as we now know it was possible on the earth.

The simplest protein, as the protein poison, has its intense chemism satisfied as it combines with other elemental groups in the development of the more complex bodies.

I began my work with the hope of finding simple proteins in the cellular structures of bacteria. In this I was disappointed and I now see that I should not have expected it. Instead of finding simple proteins in bacterial cells I have found them in the casein of milk and in the proteoses of seeds. As I have already said the young mammalian is fed upon food principles served in the simplest form. The nursing child is supplied with fats as such, with mineral constituents for the most part uncombined, with carbohydrates in the easily assimilable form of lactose and with amino acids in the relatively simple protein, casein. The sprouting seed finds the amino acids with which it starts life in the relatively simple proteins while fats and carbohydrates are supplied in a ready-made form. Now if this provision be made for the support of the developing plant and animal, what can be said about the food supplied the numerous cells of the body, whether it be plant or animal. Simple proteins exist in the circulating blood of the higher animals. Not only is this true but as Van Slyke and his coworkers have shown the body cells directly use amino acids. The simple proteins probably exist in the circulating blood chiefly in that protein mixture about which we know but little and which we designate as serum globulin. In this mixture the primitive proteins are ready to enter into combination with the more complex cellular proteins as the latter wear away in their functional activities. Their chemism is held in abeyance by combination with some indifferent substances, such as calcium. I have found that the protein poison from casein is neutralized *in vitro* by calcium lactate. Indeed the protein poison is largely, but not so quickly, neutralized by incubation with sodium bicarbonate. In this connection it may be well to recall the effect of the withdrawal of calcium on the coagulation of blood and that after severe poisoning with the protein body the clotting of the blood is retarded and often wholly prevented. If my idea that the circulating blood at all times contains the protein poison from the too violent chemism of which the body is normally protected by its combination with an inert body, it will not be difficult to understand that the equilibrium may be disturbed in a variety of ways with death as a result. The introduction of a little more of the poison or the removal of the protecting body may seriously upset the equilibrium. Casein yields about ninety per cent of its weight in protein poison. The calcium is easily removed from casein. An ash-free casein may be prepared by repeated solution in dilute ammonia and reprecipitation with dilute acid. The last trace of calcium is removed by treatment with oxalic acid. The protein poison from casein resembles the globulins inasmuch as it may be wholly precipitated from aqueous solution by saturation with sodium chloride, but differs from globulins inasmuch as it is freely soluble in absolute alcohol.

Blood is rendered poisonous not only by incubation with bacteria, agar, starch, kaolin, etc., but as was shown by Köhler as long ago as 1877, it becomes poisonous on clotting, killing both homologous and heterologous animals. This phenomenon which has been confirmed by others has recently been investigated by Moldovan who has shown that blood freshly defibrinated by shaking with glass beads causes acute death when injected intravenously into guinea pigs and rabbits. In the former the typical anaphylactic lung picture is seen after death. When the dose is slightly sublethal there is marked fall in temperature with subsequent fever. When the doses are smaller there is marked fever. On stand-



ing from fifteen to forty-five minutes defibrinated blood loses its toxicity. Serum obtained by rapid centrifugation of defibrinated blood is poisonous. The same is true of the deposited and once washed corpuscles. When coagulation is delayed by the presence of sodium citrate neither the supernatant fluid nor the corpuscles are poisonous, but both become so when coagulation is induced by shaking with porcelain beads. Doerr has shown that blood received in paraffined vessels becomes poisonous; but when the coagulation is complete the toxicity disappears. When coagulation is made to proceed slowly by the addition of hirudin solution or a 0.7 per cent solution of colloidal silicic acid, it retains its toxicity for several hours.

The fact that extracts of normal tissue, when injected intravenously, are poisonous is another interesting fact. If the lungs of a rabbit be macerated for two hours in salt solution, the solution kills promptly on intravenous injection. Homologous organ extracts are more poisonous than heterologous.

All these phenomena show that there is under normal conditions a nice adjustment in the constituents of blood and tissue whereby life is protected and that slight changes easily disturb this equilibrium with most disastrous results. There are here unsolved problems but my work leads me to the conclusions that there are protein bodies in the blood and tissue, which serve under normal conditions as cell foods, but which may become explosively poisonous when the mechanism regulating their use is disturbed. Normal cells contain deposits of these bodies, which under proper regulation, supply cell waste, but under abnormal conditions lead to cell destruction. These substances were probably present in my bacterial cells, but I washed them out and threw them away leaving only the cellular proteins. However, time and labor will solve these problems and I turn to another phase of my subject.

If I properly interpret my work on the chemistry of bacterial proteins it confirms the theoretical views of Pflüger, Ehrlich, and Verworn, who have held that the essential part of cells consists of a chemical unity, made up of giant molecules. So far as I can find this view receives additional support in the experimental work done by others. I have been able to find but little upon this subject. Reinke and Rodewald found that air dried substance of *æthylum septicum*, which they designate as *plasmodium*, consists largely of highly complex proteins containing phosphorus and yielding xanthine bases and carbohydrates on disruption. Sosnowski concludes from his study of infusorial cellular substance that this does not contain simple proteins as such, but as constituents of highly complex molecules. My studies have led me to formulate a theory concerning the nature and operation of living matter. My first attempt in this direction was made in a lecture delivered in Toronto (1905), and this was elaborated in a Shattuck lecture (1906). The cell is not the unit of life; life is molecular. Life is function, not form. The cell is not only made up of protein molecules, but its form and function are determined by the chemical structure of its constituent molecules. The lines along which the spore, seed or ovum develop are determined by the chemical structure of its proteins. Growth in other directions is impossible, and this accounts for stability in reproduction. However, changes in the chemical structure may and do occur and in these lies the basis of variation.

The keystone or archon of the protein molecule is the protein poison. It is common to all protein molecules. Physiologically it is the same in all molecules, i. e., when set free it is a poison and it is a poison on account of its intense chemism which enables it to tear off groups from other proteins. One protein differs from another in its secondary and tertiary groups. Most native proteins are not poisonous because in them the chemism of the primary group is satisfied by combination with secondary groups. Strip off the secondary groups and the primary becomes poisonous on account of the avidity with which they combine with the secondary groups of other molecules. Biological relationship between proteins depends upon the secondary groups. In this way varieties and species have developed.

The living molecule is never in a state of equilibrium. There is a constant exchange of atoms between it and the outside world. It absorbs, assimilates and eliminates. It is constantly trading in energy. It takes in oxygen and gives off carbonic acid; it takes in nitrogenous material and having utilized it the waste is discarded. The living molecule passes through the period of growth and decay. During the former its functions are largely synthetic; in the latter they are autolytic and finally the structure drops into pieces.

*(Concluded.)*

# A STUDY OF THE TESTS OF LIVER FUNCTION\*

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**I**N order to study the functional activity of an organ, it has been customary to apply certain specific tests to the individual functions of that organ. Thus if an organ has several functions, tests are applied to one of these functions, and conclusions are drawn therefrom as to the capability of the organ to perform all of its offices. This has been especially the case in the investigation of the condition of the liver.

The liver has a multiplicity of duties to perform in the body, all of which are of essential importance. It is possible that each individual cell of the hepatic structure takes part in all of the liver functions; it is also possible that different portions of the liver lobule, and different conglomerations of the liver lobules may have specific functions. In the former case, it is most likely that a reduction in the ability of the liver to perform one function will be accompanied by a proportional reduction in all the liver functions; in the latter case, one or more functions of the liver may be disturbed without affecting the other hepatic functions.

In order to appreciate the various methods for determining the liver functions, it is best to enumerate the different functions of the liver:

1. Secretion of bile.
2. Relation to carbohydrate metabolism.
  - a. Glycogen formation.
3. Relation to nitrogen metabolism.
  - a. Formation of urea.
4. Detoxification function.
  - a. Formation of the conjugate sulfates and glucuronates.
  - b. Withholding of toxins and poisons.
5. The decomposition of the erythrocytes.
6. The formation of fibrinogen.
7. The formation of antithrombin.

The methods for the study of the liver functions are several. These tests can be classified in the following way:

1. A study of the carbohydrate tolerance of the liver; this will include the tests of general carbohydrate metabolism; tests of tolerance for special carbohydrates, for example, Bauer's galactose test, Strauss's levulose test, etc.
2. A study of the nitrogen excretion in the urine, including the urea, amino, and ammonia nitrogen fractions.
3. The urobilinogen excretion in the urine, which von Jaksch, in 1892, considered significant of liver disease.
4. Analysis of the fibrinogen of the blood, which was found to disappear

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from the blood after liver extirpation (Doyon and Kareff, Nolf, Corin and Ansiaux, etc.).

5. A study of lipase and fibrinolytic ferments of the blood (Whipple, Mason and Peightal, Goodpasture).

6. The phenol tetrachlorophthalein test (Rowntree, Hurwitz and Bloomfield; Kahn and Johnston; McLester and Frazier).

1. *Carbohydrate tests of hepatic function.*

a. Bauer tested the liver function by administering to the patient 30 grams of galactose. The urine is then analyzed for galactose. If present the liver is not functioning properly.

b. Strauss used another carbohydrate—levulose—for this test. He administered 100 gms. of this substance per os and then analyzed the urine for levulose by Salmanoff's reaction.

2. *Study of the nitrogen metabolism.*

It is well known that the liver plays an important role in the protein metabolism of the animal organism. Disturbances of liver function will induce deviation from the normal of the nitrogen metabolism. Rowntree, Marshall and Chesney have found a low percentage of urea and a high percentage of amino acids and ammonia in the urine of patients suffering from hepatic involvement. It is known that in eclampsia, with liver necrosis, there is always a high ammonia coefficient.

3. *Study of the urobilinogen excretion.*

Von Jaksch thought that the presence of urobilinogen in the urine was indicative of hepatic disease. The studies of Wilbur and Addis have definitely demonstrated that such is not the case. The urobilinogen is tested for by Ehrlich's para-dimethyl-amino-benzaldehyde.

4. *The determination of the fibrinogen content of the blood* (Whipple) and of the fibrino ferment of the blood (Goodpasture) were found to be valuable by Rowntree, Marshall and Chesney.

5. *The determination of the lipase of the blood*, as recommended by Loevenhart, has not proved of any distinct value.

6. *The phenoltetrachlorophthalein test.*

In 1909, Abel and Rowntree conducted pharmacological experiments on animals with phenoltetrachlorophthalein, which was synthesized by Professor Orndorff, of Cornell University. They found that this substance, when injected intravenously, was excreted in the bile. At the suggestion of Rowntree, Whipple, Mason, and Peightal studied the excretion of this substance in the bile when the liver was subjected to artificial lesions. These authors found that in dogs which had been poisoned by phosphorus, for example, the excretion of the phthalein was interfered with. It was then that Rowntree, Marshall and Chesney applied the tests clinically and obtained rather encouraging results.

The phenoltetrachlorophthalein test is applied in the following manner:

The dye is to be prepared for use each time. One gram of the substance is placed in a 200 c.c. Erlenmeyer flask, with 2 c.c. of 2 N sodium hydroxide solution and 18 c.c. of freshly distilled water. This is boiled for twenty minutes under a reflux condenser. The solution is filtered into a 100 c.c. flask, and is ready for use. This gives approximately a five per cent solution, which is al-

most isotonic with blood. The solution is of an intense purplish color; it will not keep for more than a few days. Arbitrarily 8 c.c. of this solution, approximately 400 mgm., of the phthalein has been selected. This amount is sufficient to give a most intense purplish-red color to twenty liters of water. Its administration in health is never followed by the appearance of the dye in urine, and this amount insures in health an intense color in the final preparation of the feces, which is used for the quantitative determination. The dye is administered intravenously by gravity with antiseptic and aseptic precautions and with the usual intravenous technic. The funnel and system are filled with freshly distilled water, and after the flow is well established the phthalein solution is added. Fifty to 100 c.c. of water are used and the phthalein solution is washed in with freshly distilled water until the fluid entering the veins is colorless. Ten to fifteen minutes are required for its administration. Physiological salt solution may be preferable to distilled water for use in this injection.

Active purgation is instituted prior to the administration of the dye, and throughout the time of observation, usually by means of compound cathartic pills. The stools are collected for forty-eight hours, the urine for twenty-four hours. In the event of little or no feces being obtained, enemata are used, but unless a normal amount of dye is recovered the test must be discarded, since low findings under this condition could not be accepted.

The total forty-eight hour feces are placed in a two liter bottle and diluted with water to one or 1.5 liter, depending on their amount. This is placed in a shaking machine for from five to twenty minutes. Without allowing time for sedimentation, one-tenth of the total is placed in a one liter flask and to this is added 5 c.c. of forty per cent sodium hydroxide, which causes the mixture to take on a very red color. Dilution is made with water to one liter. A stopper is inserted and the mixture thoroughly shaken. One hundred c.c. of this preparation is placed in a 200 c.c. flask, 5 c.c. of lead acetate added, resulting in a discoloration of the mixture and a throwing out a heavy lead precipitate which carries down all the pigments, leaving a clear colorless supernatant fluid. Five c.c. of forty per cent sodium hydroxide are added; this again elicits the red phthalein color, but does not redissolve the other lead pigment combination. In certain instances 5 c.c. of sodium hydroxide at this point are not sufficient to elicit the maximum intensity of red, and more should be added until maximum intensity is reached, but not sufficient to free the other pigments from their insoluble lead combinations. The contents of the flask are made up to 200 c.c., shaken, and, a small part filtered off, or the solution is allowed to stand for five minutes, when in many cases a clear red, supernatant fluid ready for estimation can be decanted. This solution is compared in a Rowntree and Geraghty modification of the Autenreith and Koningsberger colorimeter with 20 mgm. to a liter solution of the disodium salt of tetrachlorphenolphthalein (e. g., 0.4 c.c. of original solution to one liter, plus sufficient sodium hydroxide to insure maximum color). With these dilutions the amount of dye present is indicated directly in percentages.

When the amount recovered is below normal, it is advisable to add 2 to 3 c.c. more alkali to the 200 c.c. preparation, and redetermine, thus insuring that the maximum color has been elicited. The addition of large quantities of alkali-

lies is undesirable, since it sets free the other pigments, rendering the solution yellowish-red instead of purplish-red. Not more than ten minutes are required to carry out this test after the feces are removed from the shaker. Where difficulty is experienced on account of the quality of the color, the following procedure may prove of some value in certain instances: After the addition of about 10 c.c. of forty per cent sodium hydroxide, the feces are made up with water to one liter. To one-tenth of this is added five c.c. sodium hydroxide and water up to one liter. Of this 100 c.c. are placed in a 200 c.c. flask and to it are added 5 to 10 c.c. or more of calcium chloride mixture until the best quality of color is elicited. Dilution is made to 200 c.c., the mixture is allowed to stand from one-half to twenty-four hours, and a small amount of the supernatant fluid is filtered off and read against the standard.

7. *A study of sulfo-conjugation as influenced by liver diseases.*

The cause and the location of the formation of the ethereal sulfates and of indican has been studied by a number of investigators.

Since Städeler found phenol in cow's and horse's urine, Landolt, Lieben, Hoppe-Seyler, Buliginsky and Munk found traces of it in normal human urine, and Salkowski observed that in ileus and other obstructive intestinal disease, the excretion of phenol in the urine is much increased.

This formation of phenol and phenolic substances, cresol, indol, skatol, etc., has been ascribed to the action of the intestinal bacterial flora. Such organisms like the *B. coli communis*, which is a normal inhabitant of the intestinal canal, are harmless under normal circumstances. In conditions of injury to the intestinal mucosa, these organisms become virulent (Fermi and Salto). Other organisms, like the *B. Putrificus*, *B. aerogenes capsulatus*, which are obligatory anaerobes thrive in the colon where there is no oxygen (Herter), and break up protein into the carbocyclic, toxic substances.

It was demonstrated by Baumann that these split products are very toxic, but that when they are united with sulfuric acid, they have lost their poisonous effect.

Baumann found that phenol sulfate is a normal urinary constituent and that the administration of phenol increases the phenol sulfate in the urine.

Baumann and Herter reported that not only phenol, but also other substances were excreted in the urine as ethereal sulfates. They also observed that phenol unites not only with sulfuric acid but with other radicals. This was confirmed by Schmiedeberg, who found that phenol unites with glucuronic acid.

Upon poisoning dogs with phenol, he found that the liver became rich in phenol sulfates. For example in 100 parts of liver he found 19 times as much tribrom phenol as in 100 parts of blood. This phenomenon seemed to prove that the liver is the seat of conjugation of the phenolic and indolic radicals with sulfuric acid.

Lang determined the quantity of ethereal sulfates in the urine of geese before and after extirpation of the liver. His figures are rather small, and should not be taken conclusively, but he was led to believe that the synthesis of the ethereal sulfates was not exclusively performed in the liver.

In experiments, performed in vitro, Kochs also demonstrated, so it appeared to him, that the liver was not the only seat of sulfo-conjugation. He took

liver, kidney, pancreas, thymus, muscle, and minced each organ respectively, and added phenol and disodium sulfate. He kept these mixtures at body temperature or else at 8° to 12° C. He reported that all the tissues, save the thymus, took part in the synthesis. He obtained similar results with ortho-, meta-, and para-di-oxy-phenol.

Landi repeated the experiments of Kochs, using only the liver tissue. But, as he says, due to the fact that decomposition sets in so very soon, he could not confirm Kochs' findings. In order to throw more light on the subject, he made perfusion experiments with the liver, and he came to the final conclusion that the seat of conjugation of the phenolic and sulfuric radicals was not the liver but the intestines.

The results of Landi are directly negated by the findings of Embden and Glaessner. They performed perfusion experiments on the organs of dogs, using the liver, muscle, kidneys, lungs and small intestine. From their investigations they conclude that the liver is the most important organ for the formation of the ethereal sulfates. Smaller quantities of ethereal sulfates are produced in the lungs and the kidneys, but the muscle tissue and the small intestine play a very insignificant role in the formation of the ethereal sulfates.

Reale, from his observations, was of the firm opinion that the liver was the seat of the synthesis of the ethereal sulfates.

Finizio confirmed Reale from his clinical findings. In normal individuals and in a case of echinococcus hepatic cyst, he found that the administration of thymol caused an increased excretion of ethereal sulfates in the urine. When, however, he administered thymol to a patient suffering from hepatic cirrhosis, he found no increase of the ethereal sulfates in the urine.

In normal conditions of the alimentary tract, Strauss and Philipsohn found no phenol in the urine, and they concluded that under normal conditions, the phenol and other radicals were conjugated with sulfuric acid. According to these authors, the liver is the seat of the synthesis of the ethereal sulfates.

Herter and Wakeman took 7 gms. of liver, kidney, muscle, brain, and blood respectively, which were minced, and treated each tissue with 10 c.c. of a weak phenol solution, and allowed to stand for two to three hours. The mixtures were then distilled, and they found that there was a loss in the phenol distilled over. The liver retained most of the phenol, then came in order the kidneys, muscle, brain.

In conditions of jaundice, Biernacki found four times as much ethereal sulfates as normally. Darenberg and Perroy found an increased excretion of indol and skatol in jaundiced individuals. Labbe and Vitry obtained similar results. Magrageas obtained varying quantities of ethereal sulfates in icteric patients.

Amann found that in the healthy subject there is a direct proportion between the quantities of ethereal sulfates and the total nitrogen in the urine. The coefficient of Amann may be thus expressed.

$$\text{Eth. S.} \times 100$$

$$\frac{\quad}{\text{N. Urine}}$$

The value of this coefficient varies between 1.4 and 1.5. This was confirmed by Guerbet and Rouen. Slightly smaller coefficients were obtained by Magrageas.

The question has been discussed by Eiger and Hopadze whether the aromatic compounds formed in the system are diminished in amount and destroyed under normal conditions of hepatic activity, and whether, in cases of disturbance of the function of the liver, these compounds are obviously increased and placed at the disposal of the liver for conjugation with sulfuric acid. The subject is more important in its relation to cases of disease of the hepatic parenchyma than to simply biliary stasis. The ethereal sulfuric acids are most frequently, both absolutely and relatively, increased in atrophic cirrhosis of the liver, and most markedly in tumors of the liver.

In normal urine 14 to 25 per cent of the total sulfur is present as the so-called neutral sulfur. The easily oxidizable portion of this must arise from the sulfo-cyanate of the saliva, and from other partly unknown substances, while the remainder is regarded—in part, at least—as a derivative of the taurin of the bile (Lepine). This latter bears, in the nomenclature of the French physiologists, the name “biliary sulfur of the urine.”

Lepine found, in incipient cases of obstructive jaundice in animals and in man, the biliary sulfur absolutely and relatively increased as regards the oxidized sulfur (up to 30 to 43 per cent of the total sulfur). After a few days of the biliary obstruction, the sulfur became approximately normal, and after long continuance of the disturbance showed a decrease.

Regarding the fate of taurin and the origin of the neutral sulfur in the body, the with difficulty oxidizable neutral sulfur cannot yet be regarded as the amount of formed, absorbed, and decomposed taurocholic acid. For instance, it has been shown that both components of the neutral sulfur vary within the widest limits in spite of feeding with the same amounts of food, and notwithstanding the same external relations of the animals used in the experiments, so that the special relation of the with difficulty oxidizable sulfur to taurin becomes rather doubtful (Benedict). Nevertheless, attention must be called to the fact that the early increase and later decrease of the neutral sulfur described by Lepine is very comparable to the view which we must take regarding the process of the formation of biliary acids in jaundice.

The following example, selected from Lepine's work on cholelithiasis illustrates the course of excretion of neutral sulfur in jaundice:

May 2: Light jaundice.

“ 3: Light jaundice; neutral sulfur=31 per cent.

“ 6: Sudden increase of jaundice.

“ 7: Marked jaundice, neutral sulfur=43 per cent.

“ 10: Marked jaundice; neutral sulfur=20 per cent.

Total sulfur=100.

F. Muller, who studied a case of jaundice from gall-stones of somewhat long standing, found on three days the values of the neutral sulfur to be 22.9, 15.7, and 10.7 per cent of the total sulfur. Later in the same case, but with different diet, the values were 19.2 and 17.4 per cent. In a case of carcinoma of the stomach and liver, accompanied by jaundice, the findings were 29.0, 21.1, and 16.1 per cent. These figures confirm Lepine's idea that the neutral sulfur diminishes the longer the jaundice continues.



On the other hand, a marked decrease, and even a lowering of the normal values, should be expected in chronic obstructive jaundice, provided the assumption is correct that in cases of disturbed outflow of bile into the intestine the production of biliary acids is markedly reduced by the interruption of the circulation of bile acids. Since this is not observed, the relation of the hardly oxidizable sulfur to taurocholic acid must be reinvestigated before an opinion on the formation of bile acids can be based on the excretion of neutral sulfur. Hence it does not follow that Schmidt should assume that the production of bile acids, even in long continued jaundice, suffers no reduction, because he but rarely found high values for the neutral sulfur in his case of jaundice. According to Benedict, a portion of the non-oxidized sulfur compounds, which may be excreted in increased amounts as a result of toxic action on the protein constituents of the body, are to be regarded as intermediary bodies, which resist the further oxidation to sulfuric acid. Corresponding to their presence in the bile (Bial), conjugated glycuronic acids are regularly observed in the urine in cases of biliary obstruction (van Leersum) [Von Noorden: *Metabolism and Practical Medicine*].

We studied the liver function by several of the above methods.

1. *The tetrachlorophenolphthalein test.*

We applied this test in a series of thirty-four cases. This series included patients who were suffering from liver disease, as well as those who had no hepatic ailment. The test is not as easy to carry out as the description indicates. It is rather difficult, and in many case almost impossible to impress the nurse with the importance of collecting the entire quantity of feces. The duty is rather a disagreeable one and complaints are likely to arise. The chemical analysis is also a disagreeable procedure and in a number of instances almost discouraging. In these cases it is almost impossible to obtain a color which can be compared with the standard. In general this test is not easy; it requires some experience, and it needs a well equipped laboratory.

The accompanying table shows the percentages of dye recovered in the various cases. Several cases which clinically were typical cases of liver involvement gave rather high figures for the phthalein excretion in urine, whereas other cases in which the diagnosis pointed to nonhepatic involvement, there was frequently observed a very low phthalein output.

We concluded that this test is of very doubtful value. It certainly does not lend itself to clinical purposes. It is difficult of performance; the manipulations are very disagreeable; and the results obtained not conclusive.

2. *The Urinary nitrogen partition.*

Rowntree, Marshall and Chesney concluded from their experiments that the nitrogen partition is of distinct value in determining whether or not there was a disturbance in liver function. We have obtained similar results. In eclampsia of pregnancy, where the liver is involved we found always an increased ammonia nitrogen, and amino acid nitrogen output, and a decreased urea nitrogen elimination. Normally the ammonia nitrogen is about 4 to 5 per cent of the total urinary nitrogen, and the urea nitrogen is 80 per cent of the total nitrogen. In eclampsia and in severe disease of the liver, the ammonia output may be increased to 40 per cent or more at the expense of the urea nitrogen.

NO.	NAME.	DIAGNOSIS.	OUTPUT OF PHTHALEIN, PER CENT.	REMARKS.
1	B.	Fracture	14	
2	B.	Gastritis	28	
3	B.	Fracture	31	
4	S.	Mitral insufficiency	19	
5	H.	Jaundice	5	
6	B.	Fracture	12	
7	S.	Congestion of liver	32	
8	B.	Congestion of liver	34	
9	D.	Cholelithiasis	28	
10	S.	Chronic gonorrhea	25	
11	H.	Amputation	22	
12	R.	Hernia	27	
13	K.	Renal tuberculosis	32	
14	K.	Burns	24	
15	D.	Tuberculosis	21	
16	S.	Malaria	34	
17	D.	Cholelithiasis	20	Jaundice
18	F.	Cholecystitis	21	
19	R.	Fracture	14	
20	R.	No diagnosis	23	
21	D.	Liver congestion	25	
22	H. V.	Atrophic cirrhosis	25	
23	W. J.	Atrophic cirrhosis	17	
24	E. P.	Atrophic cirrhosis	21	
25	H. C.	Syphilis of liver	24	
26	C. L.	Abscess of liver	19	
27	T. M.	Cholecystitis	25	Jaundice
28	P. A.	Cholecystitis	30	
29	R. S.	Gall-stones	18	Jaundice
30	H. O.	Gall-stones	27	Jaundice
31	W. K.	Gall-stones	26	
32	L. Y.	Cancer of liver	17	
33	H. P.	Chronic gastroenteritis	32	
34	G. L.	Mucocolitis	35	

3. *The lactose and levulose tests* have been of no value to us as an aid in the diagnosis of liver disease.

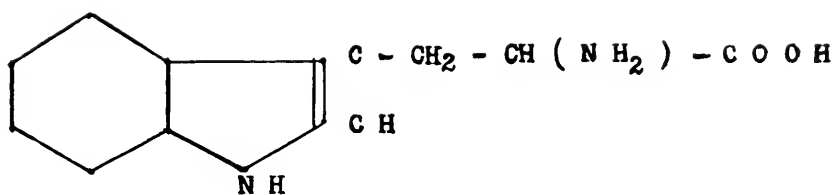
4. *The urobilinogen test* may be classed with the carbohydrate tolerance tests.

5. *The sulfo-conjugation.*

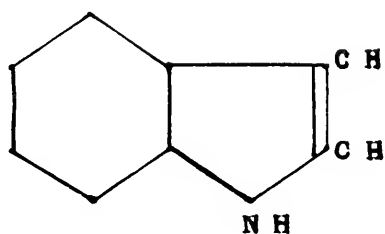
This has helped us much in determination of liver function. We shall discuss this in detail.

The toxic aromatic radicals produced by decomposition of protein are conjugated in the liver with sulfuric or glycuronic acid, and are then excreted in the urine. If we should take indol as an example, the following process would take place.

Tryptophane, or beta-indol-alpha-amino-propionic acid is one of the products of decomposition and putrefaction of proteins. It is the mother substance of indol and skatol, etc. Upon breaking down of tryptophane, indol, which is very toxic is produced.

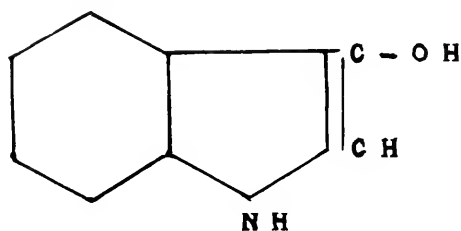


(Tryptophane)



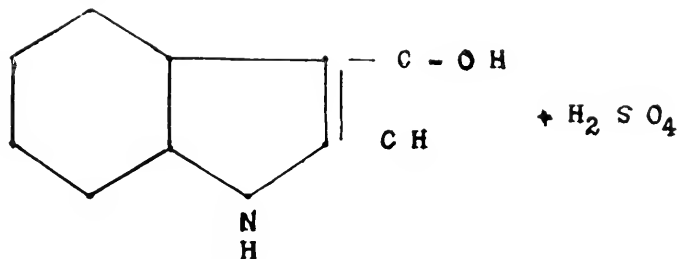
( Indole )

Indol is oxidized in the intestines to indoxyl.

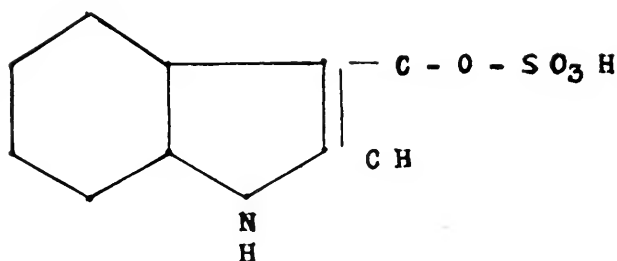


(Indoxyl)

If indol or indoxyl enters the general circulation marked toxinemia results with its concomitant symptoms. The protective mechanism of the body against this toxinemia is to conjugate the indoxyl with sulfuric acid in the liver, producing a substance which is almost nontoxic—indican.

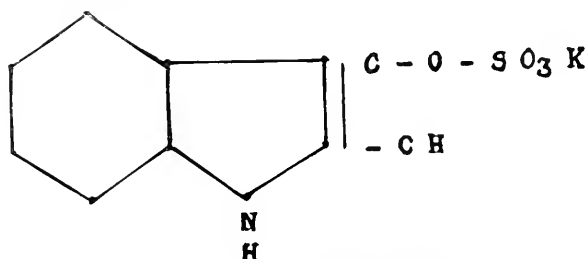


(indoxyl)



( indoxyl sulfuric acid)

In the presence of potassium salts:



(indican)

Similar results are obtained with any of the aromatic radicals, as phenol, cresol, tryosin, skatol, etc.

It is well known that the total sulfur in the urine may be partitioned into three distinct fractions:

- a. The Inorganic Sulfates.
- b. The Ethereal Sulfates.
- c. The Neutral Sulfur.

It has been definitely established that, normally, the inorganic sulfates form about 70 per cent of the total sulfur, and the remaining 30 per cent are divided almost equally between the ethereal sulfates and the neutral sulfur.

The ethereal sulfates are the conjugated aromatic sulfonic acids. It is this fraction that is of special interest to us now.

It is, of course, impossible to rely upon the excretion of ethereal sulfates as a symptom of hepatic function. The proteins which are ingested daily give rise to their quota of aromatic radicals which influence the quantity of the conjugated sulfates. The condition of the intestinal flora plays a role in the formation of aromatic radicals, thus it is known that in intestinal putrefaction there is a marked increase in the conjugated sulfates excreted.

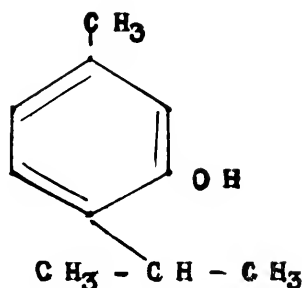
We, therefore, adopted the following technic for the determination of liver function by means of the ethereal sulfate output:

The patient received a dose of castor oil to clean out his bowels. He was then kept on a known diet for two days, during which time the urine was collected, preserved, and analyzed for total sulfur and ethereal sulfates\*. On the

\*The total sulfur was analyzed by Benedict's method; the Ethereal sulfates by Folin's method.

third day the patient received a capsule containing one-half gram of thymol. The urine was collected for the next two days, preserved, and analyzed for total sulfur and ethereal sulfates.

Thymol is iso-propyl-meta-cresol:



If all the thymol were absorbed and if all the thymol were conjugated with sulfuric acid and none with glycuronic acid, the 0.5 gram of thymol would be excreted as 0.7666 gms. of thymol sulfuric acid. This would cause a marked increase in the percentage of ethereal sulfates. If the liver were not functioning properly, the thymol would not be conjugated, and the percentage of ethereal sulfates would be only slightly different from what it had been on the first two days.

One objection to the study of the function of any organ as an index of disease of that organ, is, that it is perhaps possible for the healthy part of the diseased organ to compensate and assume the work of the whole gland. In such a condition of course the functional output of the organ may be normal, and would be no index of the pathological anatomy of the organ. Under these circumstances only marked destructive changes would leave their impress on the functional activity of the organ.

#### ETHEREAL SULFATE ELIMINATION BEFORE AND AFTER THYMOL ADMINISTRATION.

Case No.	Diagnosis.	Total Sulfur gms.		Ethereal Sulfate Sulfur gms.		Ethereal Sulfate Sulfur % of Total Sulfur.	
		Before Thymol.	After Thymol.	Before Thymol.	After Thymol.	Before Thymol.	Thymol. After
1	Normal	2.0375	2.1295	0.2893	0.5646	14.2	26.8
2	Gastritis	1.9428	1.7427	0.1457	0.3380	7.5	19.4
3	Fracture	2.7467	2.5527	0.3131	0.6024	11.4	23.6
4	Congestion of liver	0.9852	1.0734	0.1753	0.7069	17.8	28.6
5	"	1.7345	1.6982	0.2480	0.3610	14.3	21.2
6	Gall-stones	2.7628	2.8075	0.7597	1.0303	27.5	36.7
7	" "	3.0042	2.6826	0.3965	0.8474	13.2	29.4
8	Cholecystitis	2.7807	2.6437	0.4866	0.7428	17.5	28.1
9	Atrophic cirrhosis	2.2328	2.3029	0.2791	0.3400	12.5	15.2
10	Tumor of liver	1.9492	1.8757	0.1637	0.3676	8.4	19.6
11	Cancer of liver	2.7526	2.6278	0.6083	0.6648	22.1	25.3
12	Syphilis of liver	2.8104	2.9075	0.3990	0.5437	14.2	18.7

It has been our experience, however, that disturbances in the structure of the liver goes hand in hand with disturbances of function, especially as is indicated by sulfuric acid conjugation of the aromatic radicals. We have found that in cirrhosis of the liver the conjugation of thymol with sulfuric acid does not take place to as marked an extent as in the normal state. This question is now being more fully investigated, and in the very near future we hope to make a more extensive report. Meanwhile, we have cited a few cases above.

It will be observed that in the nonhepatic diseases, and in the nondestructive diseases of the liver, a marked increase in the excretion of ethereal sulfates was observed on the day after the thymol administration. In diseases of the liver, like atrophic cirrhosis, cancer of the liver, or syphilis of liver, this organ has lost its power to conjugate the thymol with sulfuric acid. Case number 10 was a benign tumor of the liver, and it seems no destructive changes went on in the hepatic tissue. This case was in the service of Dr. E. B. Haworth.

We hope to study this reaction more fully in experimental hepatitis, if possible.

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## SOME TECHNICAL DIFFICULTIES INVOLVED IN THE COMPARISON OF THE DIAZO AND UROCHROMOGEN TESTS\*

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SINCE the publication of Weisz<sup>1</sup> urochromogen test an abundant literature has developed, a very striking feature of which is the wide difference of opinion as to the relative value of the diazo and urochromogen reactions. Weisz found the urochromogen reaction more frequent than the diazo and recommended it as a more sensitive substitute for the latter. He did not give any tabulated comparisons of the two tests. Later workers have tabulated their experiences. Heflebower<sup>2</sup> found the diazo positive in only 29.1 per cent of a series of 72 cases, while the urochromogen was positive in 66.6 per cent. Gullbring,<sup>3</sup> in a series of 168 cases, found the diazo positive in but 28, while the urochromogen was positive in 88. Sinclair<sup>4</sup> reported 148 cases of which only 30 gave the diazo, but 85 gave the urochromogen. Schaeffle's<sup>5</sup> series of 190 cases showed a much closer relation between the two tests,—40 giving diazos, and 51 giving urochromogens. Other workers have reported values between these extremes.

The differences in percentage incidence of diazo and urochromogen in the above reports, indicate either a striking selection of certain types of tuberculosis among the institutions from which the reports were made, or a lack of a uniform technic and criteria for deciding the presence or absence of the reactions.

My early experiences failed to show such remarkable sensitiveness of the urochromogen as compared with the diazo, as was reported by the first group of workers, and it seemed that this difference might be explained, at least in part to variations in technic, due to the rather indefinite instructions of Weisz. His simple instructions are: Dilute the clear urine with two volumes of water and add three drops of a solution of potassium permanganate (1:1000). A yellow color indicates the presence of the urochromogen body. These instructions were considered sufficient,—in marked contrast to the care necessary for performing the diazo test.

Various workers have attempted to standardize certain features of the technic, thus Heflebower used 1 c.c. urine and 2 c.c. of water. Schaeffle "recognized as a positive reaction only a distinct canary yellow which remained fairly permanent, although there were frequent slightly yellow reactions which soon disappeared." Heflebower on this point said: "The appearance of a yellow color shows that urochromogen is present." With these, and a few other exceptions, however, no serious attempt appears to have been made to standardize the urochromogen test. Most workers have been silent on the details of technic.

According to Weisz' instructions, it is evident that in practice two workers might employ widely different proportions of reagent and urine in perform-

\*From the Pottenger Sanatorium for Diseases of the Lungs and Throat, Monrovia, Calif.

ing the test,—three drops ranging anywhere from 3/15 to 3/30 c.c. and the quantity of urine used from 3 c.c. to 6 or 7 c.c. In this manner, one worker, by adding 3/30 c.c. of reagent to 6 or 7 c.c. of urine would employ less than 1/4 the proportion of reagent to urine as the other worker who adds 3/15 c.c. of reagent to 3 c.c. of urine.

The following method was employed to determine, first, the differences in color tone and permanency of reaction due to various proportions of urine and reagent; second, the relation existing between the color tones of foam and solution in the diazo and the urochromogen; third, the influence of the age of the specimen upon the test; and fourth, the conditions for the standardization, if found advisable, of the urochromogen reaction. The regular twenty-four hour specimens from patients, and fresh morning specimens from both patients and normal persons were examined. At least one normal urine was placed among the urines to be analyzed, the identity of all specimens being concealed until the tests were made. The work here reported has been carried out over a period of one year.

The records were kept in tabular form, of which the following is one.

TABLE I.

DETAILED UROCHROMOGEN RECORD, INDICATING COLOR CHANGES AT DIFFERENT TIME INTERVALS, WITH DIFFERENT PROPORTIONS OF REAGENT USED AND AT DIFFERENT DILUTIONS.

	1/3 dilution.					1/6 dilution.					1/10 dilution.				
	Initial	15 s.	30 s.	60 s.	120 s.	Initial	15 s.	30 s.	60 s.	120 s.	Initial	15 s.	30 s.	60 s.	120 s.
0.25 c.c.	y-br ++	++	++	++	++	br-y +	+	+	+	+	y-br	y?br-	br	+	+
0.15 c.c.	y-r ++	++	++	++	++	y	+	+	+	+	br-y	lbr-y	ly-br	+	+
0.10 c.c.	y	+	+	+	+	ly	+	+	+	+	ly	+	+	+	+
0.05 c.c.	ly	+	+	+	+	ly	+	+	+	+	ly	+	+	+	+
	Control	br-y	++			lbr-y	+				ly-br	+			

In making the urochromogen test, 5 c.c. of the 1/3 dilution of urine was placed in each of 5 test tubes, selected so that the column was of uniform depth. In case the test was positive, further dilutions were made, say 1/6, 1/10, 1/15, etc., until the test failed to appear in one or more tubes. Using one tube as control, to the other tubes were added in succession, .25 c.c., .15 c.c., .10 c.c., and .05 c.c. of potassium permanganate (1:1000), by means of a pipette graduated in twentieths. This series corresponds to 5 drops, 3 drops, 2 drops and 1 drop, when 20 drops equals one cubic centimeter. The color change was recorded at once, at the end of 15 seconds, 30 seconds, 60 seconds, and 120 seconds by reading through the end of the tube held against a white background. The colors viewed from the end of the tube are intensified, as compared with the same viewed laterally, and slight reactions will be found by the first method, where they would be considered negative or questionable by the second. On the other hand, where the urochromogen is strong, the appearance viewed from the end is misleading, especially in the 5 drop and 3 drop tubes, the brown tone



predominating; but, if viewed laterally, a deep pure yellow is present. There is no likelihood of confusion, however, on this point. It must be pointed out, however, that my readings contain a more pronounced brownish element than they would, had they been made viewing the tubes laterally. Quantitative differences were indicated roughly by employing the terms, light yellow, yellow, light brown, brown, and their combinations, and by affixing the usual signs | +, +, ++, +++.

The diazo tests were made with solutions according to the original formulæ of Ehrlich. Solution A, Sulphanilic acid 5 gms., Hydrochloric acid 50 c.c., and water 1000 c.c.

Solution B, Sodium nitrite 0.5 per cent. The test solution was made fresh by adding B to A in the proportion of 1:50. Exact quantities of urine and test solution were measured by pipette into a small 3 inch test tube,  $\frac{1}{10}$  volume of ammonia was added and shaken. Both foam and solution were described at once. In positive cases the diazo was performed on a series of dilutions of urine in the following table:

TABLE II.

DETAILED DIAZO RECORD ON SAME URINE AS WAS USED IN TABLE I, SHOWING CHANGE IN COLOR TONES DUE TO DIFFERENT PROPORTIONS IN THE URINE-REAGENT MIXTURE.

## Urine Solution.

$\frac{1}{2}$ vol. Reagent +	1 vol. R. +	1 vol. R. +	1 vol. R. +	1 vol. R. +	1 vol. R. +	1 vol. R. +
1 vol. Urine	1 vol. U.	1 vol. U/2	1 vol. U/3	1 vol. U/5	1 vol. U/7	1 vol. U/10
Foam						
L br	Pink	Pink	Pink (?)	White	White	White
Solution						
Red-br	Red	Br-red	Red-br	Red-br	Y-br	Lbry.

Studies were made on 60 fresh morning specimens from apparently normal persons and on 121 from patients, also on 226 twenty-four hour specimens from patients.

TABLE III.

SUMMARY OF UROCHROMOGEN TESTS MADE ON 58 NORMAL SPECIMENS, SHOWING COLOR REACTIONS AND THEIR PERMANENCY IN RELATION TO THE PROPORTION OF REAGENT USED.

Quantity of Reagent	Initial Reaction		15 seconds		30 seconds		60 seconds		120 seconds	
	Yellow	Yellow?	Yellow	Yellow?	Yellow	Yellow?	Yellow	Yellow?	Yellow	Yellow?
0.25 c.c.	39	5	5	5	1	1				
0.15 c.c.	38	2	2	2	1	0				
0.10 c.c.	19	4	1	0						
0.05 c.c.	8	9								

In two of the above urines with specific gravity of 1011 and 1006, the 5 drop tubes contained permanganate in excess, which gave a purple color, persisting for 8 or 10 seconds. The complete absence of yellow in all tubes was obtained in but 18 specimens.

The preceding table shows that yellow reactions occur in the majority of apparently normal urines, a few of which persist for some time depending upon the amount of reagent used. These normal, or pseudo reactions probably depend upon the presence of oxidizable substances other than the urochromogen body, from which, in practice, they cannot be differentiated by color tone. As, however, they are more or less transient, passing into a pure brown of varying degrees of intensity, the time of their duration is very important in excluding them from the typical urochromogen reaction which is much more permanent even when very slight. Since the yellow had disappeared in 30 seconds in all but one and possibly two of the above specimens, special attention was paid to the history of the persons furnishing them. No reason could be found for excluding them from the table.

In order, then, to exclude normal reactions, I have determined on the following standards of time for observation. Five and three drop tubes, 60 seconds; two drop tube 30 seconds; one drop tube 15 seconds.

TABLE IV.

SUMMARY OF COLOR REACTIONS IN FOAM AND SOLUTION OF DIAZO TESTS IN 60 NORMAL URINES.

	Foam.	Solution.
Colorless	48	
Yellow		40
Brown	12	18
Reddish?-brown		2

Two of the above urines giving questionable reddish tones in the diazo solution were excluded from Table III, although, in neither, did the yellow persist to 30 seconds in the urochromogen test. I have never seen a red tone, or even a suggestion of red in a diazo solution in a strictly normal person. One of these persons had been drinking heavily, though otherwise apparently healthy; the other had had tuberculosis years ago, with apparent recovery, and of late years has suffered from chronic constipation, and has shown at all times considerable anemia.

TABLE V.

SUMMARY OF UROCHROMOGEN REACTION IN 167 TWENTY-FOUR HOUR SPECIMENS AND 94 FRESH MORNING SPECIMENS FROM PATIENTS WHO DID NOT SHOW A RED COLOR IN EITHER FOAM OR SOLUTION OF THE DIAZO TEST.

Quantity of $KMnO_4$	Initial Reaction.		15 sec.		30 sec.		60 sec.		120 sec.	
	Y	Y?	Y	Y?	Y	Y?	Y	Y?	Y	Y?
0.25 c.c.	146	61	11	28	1	2				
0.15 c.c.	141	38	6	21		1				
0.10 c.c.	126	32	5	8	1		1		1	
0.05 c.c.	44	43	3	3	2		2		1	

Five drops was found to be excessive in 17 of the tests made. Fifty-three did not show yellow at all in any of the tubes. The results on both twenty-four hour and fresh specimens are combined, as no essential difference could be determined between the separate tables. The twenty-four hour specimens, however, gave a larger proportion of questionable reactions.

Comparing the above with Table III no essential difference is evident in the 5 drop and 3 drop series. The 2 drop series in the above shows a striking increase in initial reactions. The 2 drop and 1 drop series show a slight tendency to persist beyond the time standard determined from Table III. The 2 drop tubes show 1 positive urochromogen and the 1 drop, 2 positive urochromogens, which the 5 drop and 3 drop tubes fail to show. From this it is clear that 5 drops and 3 drops are excessive, resulting in a rapid change to brown. The action of excess of reagent is further shown in Table I, dilution 1/10, where the 5 drop and 3 drop series become rapidly brown, while the 2 drop and 1 drop series give a slight initial yellow developing in intensity to 15 seconds, and then persisting almost without change for 2 minutes. The result is a negative reaction in the 5 drop and 3 drop tubes, although the 2 drop and 1 drop tubes give a splendid positive reaction. This result occurs invariably where a urine giving a positive test is sufficiently diluted.

It is evident then that 5 drops and 3 drops of reagent to 5 c.c. of dilute urine is not the optimum proportion in which to make the urochromogen test. Summarizing the objections to these quantities we find,—(1) they are excessive, particularly the former, for some urines, imparting the purple color of the reagent to the mixture; (2) they intensify the initial reactions and increase the difficulty in differentiating from normal reactions; and, (3) slight reactions are overlooked due to rapid development of brown color tones.

It is interesting in this connection that Heflebower in the article cited, used his reagent—urine mixture in the same proportion as the 5 drop—5 c.c. urine mixture above. He used a 3 drop—3 c.c. mixture. Of the many workers who have published reports, he has given the most complete statement of technic.

While it is possible that Weisz and others following him have made the reagent-urine mixture in the optimum proportions, i. e., 1 to 2 drops to 5 c.c., his original statement of technic is inadequate; and, in order to guard against error in technic and interpretation, the technic should be standardized.

#### STANDARDIZATION OF THE TECHNIC FOR THE UROCHROMOGEN REACTION.

Put 5 c.c. of 1/3 dilution of urine (1 part U. to 2 parts water) into each of two test tubes, selected so that the column of liquid is 3.5 cm. deep. Hold over a strong white background and place 0.1 c.c. potassium permanganate (1:1000) in one of the tubes. Holding the tubes vertically, and looking into them from the end, record any increase of yellow at the end of 30 seconds as a positive urochromogen reaction.

In the following table D+ is a positive test with pink foam. D+? is a questionable reaction, in which the color is suggestive of pink, but uncertain. In practice, it has been my custom to call D+? reactions "suggestions" and almost invariably when found, if an afternoon specimen is called for from the same patient a distinct diazo will be found. U+? is a questionable urochromogen reaction. Although a red or reddish solution is not accepted as a proper criterion for determining the presence of the diazo, because of the fact that many known substances and some drugs are known to give similar reactions with the reagent, I have had no reason to suspect confusion on this point, and while I

have accepted the standard criteria in determining the presence of a diazo, I have long suspected that we have missed much that the reaction offers us because we have failed to observe the color tone of both foam and solution. My experience with the diazo reaction extends over eight years, having found it about 2,000 times in 8,800 twenty-four hour specimens from approximately 1,500 tuberculous patients.

TABLE VI.

COMPLETE SUMMARY OF DIAZO AND UROCHROMOGEN TESTS FROM 738 SPECIMENS INCLUDING 391 IN WHICH ONLY THE 5 DROP AND 3 DROP PROPORTIONS WERE USED, FROM 183 PATIENTS.

Color of the Diazo Solution	D-	D+	D+?	U-	U+	U+?
Red	16				16	
	3					3
	4			4		
		115			115	
		2				2
		2		2		
			9		9	
			1			1
			4	4		
Red ?	9			9		
	3				3	
	1					1
Deep brown- ish yellow	2				2	
	1					1
Yellow	2					2
	1				1	
Canary	1					1
Totals:	43	119	14	19	146	11
D- and D+? transferred to D+	20	156	0	19	146	11

The table gives 119 diazos and 146 urochromogen reactions. But, if we add to the positive diazos, the 23 D— reactions with a distinct red tone in the solution, we have a total of 156 reactions which would be balanced by the addition of questionable urochromogen reactions, making 157. However, there are still 13 abnormal diazo reactions in which a questionable reddish solution was found, the predominant tone being brown or yellow, or both. The foam in these cases was usually a much deeper brown than is found in normal reactions. If these thirteen abnormal reactions are added to the 156 reactions, we have a total of 169 abnormal diazo reactions, as compared with 157 urochromogen reactions.

In making the diazo and urochromogen tests on various dilutions of urine, illustrated by Tables I and II, another source of confusion in comparing the tests was discovered. In several instances, with a positive diazo, the urochromogen was recorded either negative or questionable at  $1/3$  dilution, while dilutions of  $1/5$ ,  $1/6$ , etc., gave unquestionable reactions. There were four

specimens, all deep yellow in color, giving U— reactions, at 1/3 dilution, but at 1/6 dilution two of them were distinctly positive and the other two were questionable in the 2 drop and 1 drop tubes. This difficulty was met with only in concentrated, particularly deep yellow urines. Brownish urines gave no trouble on this point. From this it is clear that some urines should be diluted more than 1/3 as Weisz recommended, in order to demonstrate the reaction.

Comparisons were made between tests on fresh specimens and on the same specimens after twenty-four hours standing at room temperature. No important differences were found between the amounts of the diazo and urochromogen bodies in the two examinations. Certain urines, however, giving U— reactions when fresh, gave U+? reaction at the end of the twenty-four hours. This was attributed to the development of brown color in the specimen, due to light, so that extremely slight reactions would be noticed, which were overlooked in the fresh yellow specimens.

#### CONCLUSIONS.

1. The remarkable sensitiveness of the urochromogen reaction as compared with the diazo reaction, reported by some workers, is due in large part to failure to exclude the normal transient reactions found in normal urines and to a hesitancy in recording slight diazo reactions.

2. The urochromogen is somewhat more sensitive than the diazo if the pink foam alone is considered in determining the presence of the latter; it is equally sensitive to the diazo if, compared with all reddish reactions in the solution of the latter, and less sensitive than the diazo if all questionable reddish solution reactions and those with deep brown foam are added to the latter.

3. The difference in color tone and permanency of reactions affected by various proportions of reagent and urine in performing the urochromogen test makes it imperative that the test be standardized.

4. The diazo reaction, if studied carefully as to color tone of both foam and solution, will give us considerably more information than the urochromogen gives.

5. In view of the prevailing confusion in the matter of technic, it seems too early to draw conclusions as to the relative prognostic values of the two reactions.

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## CASES OF INFECTION TERMINATING IN SEPTICEMIA\*

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ONE has always to differentiate between primary and secondary infections, just as one must recognize localized and generalized (*septicemic*) infections. One may take as a type of severe primary infection erysipelas which may or may not be septicemic. So long as it is localized, even though it is spreading, it is not a particularly serious affair, but let the organisms gain entrance to the blood stream, and it becomes a very serious menace to the life of the individual. Erysipelas may also be secondary, but the usual cases one sees are primary in the usual sense of the word. On the other hand, one may consider a case of lobular pneumonia appearing as a sequel of diphtheria. The pneumonia may be the result of lowered resistance caused by the attack of diphtheria, or it may be the result of aspiration of materials from the larynx during a period of partial asphyxia caused by the presence of the laryngeal or tracheal membrane. In either case the pneumonia is secondary whether or not it be caused by the diphtheria bacillus or some other more frequent pulmonary invader.

Again we recall the frequent terminations seen in connection with cases of nephritis in which the final stages of the life of the patient are marked by appearance of friction rubs in evidence of pleuritis or pericarditis, or the intestinal upsets indicative of the too frequent pseudomembranous enteritis. Here we are dealing with what has been called a *terminal infection* which, in the reduced state of the patient's resistance, overwhelms him and hastily carries him off. Again, when in case two or more organisms appear in a lesion we speak of *mixed infection* by which we often mean that we are not able to determine which of the germs present was the primary invader.

The following histories illustrate well a certain type of infection and also they illustrate well the organic and tissue changes produced by two organisms, streptococci and staphylococci, in septicemia.

### CASE I.

The first example to be offered exemplifies the type of severe, essentially primary, streptococcus infection, which became a mixed infection later, but which evidently was the immediate cause of death. In this case, as is frequently the case, there was no demonstrable localization of the organisms which, while they possibly had their nidus in the mouth or pharynx, gave no definite indication of such a localization but proceeded to grow rapidly and invade the blood stream by which they were generally distributed.

A. R., Hospital No. 1005, was admitted to the Cincinnati General Hospital on April 6, 1915. He died on April 12 of the same year.

### CLINICAL NOTES.

*Complaint.*—Pain, which at times is sharp, in the epigastrium and in both hypochondriac regions.

\*From the Pathological Institute of the Cincinnati General Hospital and the Mary M. Emery Department of Pathology of the University of Cincinnati.

*Family History.*—Irrelevant.

*Past History.*—The patient stated that he had not had the common diseases of childhood. Seven years before admission he had been operated upon for a left inguinal hernia. He denied venereal disease, but said he smoked and chewed tobacco and drank beer and whisky to excess until three months before admission.

*Present Illness.*—Nine days before admission he ran up a flight of stairs and became suddenly dyspneic. He went home and shortly thereafter developed the pain of which he complained. For seven days he had been aware of the rapid beating of his heart. For this he had seen two physicians who told him to go to bed and remain there. He was not able to have proper care at home and came to the hospital.

*Present Condition.*—The patient was a white man, young, well-developed and well-nourished. On entrance his temperature was 98.6, pulse 168, respiration 48. He was dyspneic, had a very rapid heart, and pain in the upper part of the abdomen. The pupils reacted to light and during accommodation. The conjunctivæ were clear. His teeth were part false; those remaining, and the gums, were in poor condition and evidently neglected. The neck was not evidently abnormal. The chest was well developed; the right side was more prominent than the left. Anteriorly the percussion note was somewhat hyperresonant; posteriorly, at the right base, the resonance was diminished, and the tactile fremitus reduced. Auscultation showed that the breath sounds were roughened, with piping râles over the left, and the mucous râles from the scapular angle to the base on both sides posteriorly. The heart was evidently enlarged. Dullness was marked in the right second interspace, and the whole right border of the heart extended beyond the normal line. Also, the area of cardiac dullness extended to the line of the left nipple. The heart rate was rapid (168) but the rhythm was regular, and at the systolic orifice there was evidence of roughening of the sounds. The lower border of the liver was palpable below the costal margin. There was no abdominal tenderness, distension or rigidity. In the left inguinal region was the scar remaining from the old herniotomy. There was no edema of the extremities. The genitals were not abnormal. Upon the back there were a number of macules showing a tendency to become papular.

The third day after admission (April 9) the patient was cyanotic, and the apex beat was plainly visible. On auscultation moist râles were heard everywhere, especially over the left lung. The pulse rate was 156. Ten ounces of blood were let by venesection. The following day the cyanosis was less evident, and there were numerous hyaline casts in the urine. On the next day the general condition was worse; there was edema of the lower extremities, severe cyanosis. The urine showed abundant hyaline casts. On the following morning the patient died.

*Clinical Diagnosis.*—Acute cardiac dilatation; mitral insufficiency; embryocardia; acute nephritis.

#### AUTOPSY PROTOCOL.

The body was that of a young adult, well-built and well-nourished white man, 5 feet, 8 inches long, and apparently about 30 years of age. He had a scanty beard. The pupils were equally and moderately dilated. Rigor mortis was present, and postmortem lividity was vivid. The whole body, especially the upper half was tremendously cyanotic, and scattered over the body, but especially over the thorax, particularly over the back, were numerous small hemorrhagic lesions, most of them drying and crusted, which in some respects resembled the lesions of a malignant scarlet fever. On the outer surface of the right arm, at a point about the middle, was a subcutaneous nodular mass that had something the appearance and feel of a lipoma, but which seemed to fluctuate, and which, on section, proved to be an abscess filled with a thick greenish-yellow pus. Smears showed staphylococci present and cultures proved these to be the *Staphylococcus pyogenes albus*. When the body was turned on the side, a large amount of clear, sanguineous fluid ran from the nose and mouth, and this was followed by a clear thin mucoid fluid containing material which looked like coffee grounds.

The face had a generally swollen appearance as had the whole body, and this was especially marked in the neck. There seemed to be evidence, to the eye, of a general edema, and yet there was very little pitting on pressure but only local pallor. The neck and the back of the head, including the ears, were a deep purple color. On the inner aspect of the left elbow was a wound caused by a hypodermic needle. Seven cm. medianly from the left nipple was another punctured wound (hypodermic) which led to an area of hemorrhage in the great pectoral muscle. There was the slightest edema of the legs. The peripheral lymph glands were palpable but not enlarged remarkably.

The median section showed that the muscles and subcutaneous tissues were exceedingly edematous and that the greenish colored fat was the seat of a serous infiltration. In the abdomen there were 600 c.c. of a cloudy serous fluid. The diaphragm reached to the lower edge of the 6th rib on the left and to the upper border of the 6th rib on the right. Both pleural cavities were filled with a clear sero-sanguineous fluid. Just beneath the ensiform and reaching a centimeter or more below it, was a mass of tissue which in general structure resembled jaundiced salivary gland tissue. Part of it also resembled to the eye, lymph glandular tissue. Upon investigation it was found that this tissue was a part of a mass of tissue which ran upward into the thymus region and that it was continuous with what seemed to be the thymus itself. In the thymus region there was no distinct line to be seen between thymus tissue and adiposa, (because of hemolytic changes) but about the trachea and in the upper mediastinum, this tissue formed a mass that weighed perhaps 50 grams. From it there ran down over the pericardium, a wide, thin mass of the same tissue, which divided at about the upper end of the area of absolute cardiac dullness to run laterally on either side, parallel to and just beneath the median edges of the lungs to the diaphragm when again the volume of the tissue was increased. This tissue and all the mediastinal tissues were exceedingly edematous and gelatinous in appearance. In the thymus-like material which completely surrounded the trachea just above the bifurcation, the tissue was infiltrated diffusely with a thin sero-purulent fluid which contained capsulated Gram-positive diplococci, short chains of streptococci and small clumps of staphylococci. There were no fusiform organisms nor spirochetes present. The trachea, larynx, tongue and tonsils were removed *en masse*, and except for edema of the perilaryngeal tissues, showed nothing except an intense congestion and cyanosis. This was so severe that the thyroid appeared to be the seat of an acute inflammation. The peritracheal and bronchial glands were hyperplastic and moist. The tonsils showed no evident abnormality other than atrophy. The teeth were not exceedingly bad, but there were some bridges and the upper incisors were false.

When the thorax was opened, the lungs did not collapse. Over the middle third of the upper left lobe, on the pleura, was an area measuring roughly 4.5 cm. in diameter, which was congested and the surface of which was covered with a scanty recent fibrinous exudate. Again, over the middle third of the anterior half of the left upper lobe was a similar but less definitely defined area of the same character. The upper four-fifths of the left upper lobe was almost completely consolidated, and on section had the appearance of a massive red hepatization modified by unusual edema, and hemolysis. The blood vessels leading to this area were all patent. Except that the process was less advanced, the right lung showed the same general appearance. (Weights, left lung, 1750; right, 1450.)

The pericardium contained an increase of cloudy serous fluid. There was a large edematous milk spot over the left ventricle near the apex, and a small one in the usual place over the right ventricle. In the pericardium over the base, were very numerous petechiæ. The heart was large, but was not weighed. The myocardium was only moderately hypertrophied but the cavities were much dilated. The orifices also were dilated and in this the pulmonary participated. The myocardium was pale and evidently the seat of fragmentation. The valves were not abnormal. There was no essential thickening and no vegetation on any leaflet or segment. The aorta was smooth and not sclerotic. The endocardium, including the valves and the aortic intima, was stained with the products of hemolysis. Between the columnæ carneæ of the left ventricle were numerous globular mural thrombi all of them with softened grumous centers.

The spleen (230 grms.) was adherent by old adhesions to all the surrounding organs. It was slightly enlarged, firm, moderately edematous, uniformly grayish-brownish red in color, not softened and the glomeruli were not distinct.

The liver (2116 grms.) was enlarged. The anterior surface showed a well marked capsular thickening, so diffuse that only occasionally could one see the color of the liver substance through small clefts in the hyaline membrane. On cross section the organ showed a typical nutmeg appearance.

The pancreas was slightly congested but otherwise not apparently abnormal.

The kidneys were large (right, 225 grms.; left, 295) and moderately congested and extremely cloudy. The capsules removed with ease for the most part, though the cortex was torn in a few places. The stellate veins were only occasionally dilated. The line of demarkation between cortex and medulla was only moderately distinct. The cortex and medulla were both congested, the medulla rather more so. The glomeruli were not distinct. The cortex was thickened (9 mm.) The pelvis and ureters were normal.



The adrenals showed nothing remarkable other than congestion. They were of normal size.

The mesentery was quite edematous and on section had somewhat the same appearance as the mediastinal (thymus?) tissue. The lymph glands were pale and juicy, but not hyperplastic.

The large bowel was filled with firm, rounded fecal masses. The appendix appeared to be normal. There was no abnormality of position of the abdominal organs. The stomach was filled with a large amount of thin fluid, dark grayish-green in color, in which were large solid coagula of milk. There was a moderate grade of chronic catarrhal gastritis evident. The gall ducts were patent. The papilla of Vater was apparently normal. The gall bladder contained a fair amount (50 c.c.) of a mucoid brownish-green bile with no stones or sand.

Nothing abnormal was observed in the small intestines, but in the large bowel the lymph follicles were diffusely enlarged and congested.

The genital tract seemed normal.

The brain (1625) showed intense congestion of the pial vessels together with a marked edema. There was no evidence of meningitis. The spinal fluid was increased in volume. There were no other lesions observable. The pituitary appeared to be normal.

*Anatomic Diagnosis.*—Cervical (peritracheal) and upper mediastinal cellulitis; Septicemia; Generalized edema; Cardiac hypertrophy; Cardiac thrombi; Cardiac dilatation; Acute pseudolobar pneumonia; Pulmonary edema; Splenic and hepatic hyaloserositis; Passive congestion of the liver, lungs, kidneys, stomach, intestines and spleen; Acute pleuritis; Abdominal pleural and pericardial effusion; Chronic and acute diffuse nephritis.

In this case we seem to see an illustration of the malignant course of an infection which progressed without any definite localizing onset. The clinical history is brief, to be sure, and perhaps imperfect. There is no history of sore throat nor of pain in the neck. The teeth were in a foul condition beneath the bridges. The infection commenced insidiously, causing cyanosis and epigastric pain. Even at this time the myocardium was evidently damaged, and then later came the evidences of pulmonary and renal involvement. The main organism in this was evidently the streptococcus longus or hemolyticus. The staphylococcus was a secondary invader, possibly from the skin lesion on the arm, and the gram-positive diplococci possibly of pulmonary origin, and may be looked upon as producing a terminal infection.

#### CASE II.

This case is a typical one due to pure infection with the *Streptococcus hemolyticus*.

#### CLINICAL NOTES.

W. P., a white man aged 71, was admitted to the Cincinnati Hospital on July 8, 1916. He died on July 10, 1916. At the time of admission he had a normal temperature, though the face was marked with the erythema of early erysipelas. He gave a history of having been drinking a good deal. At the time of admission his lungs were clear. The heart rate was increased, and the heart was slightly enlarged. There was a definite accentuation of the second mitral sound. That afternoon and the following day the temperature became elevated, and there was some slight roughening of the breath sounds over the upper part of the left lung, but no râles were heard. During the day the temperature remained high and the patient became restless. At the same time the face became more swollen and the inflammation appeared to be extending to the neck which showed some erythema. On the following day at 8 o'clock a. m., the temperature was 101.8 F., the respirations were increased, the neck was swollen and the inflammation had extended toward the thorax. At this time the patient commenced to have difficulty in swallowing. Also, the respiratory sounds over the upper left chest were more roughened and there were a few moist râles. At 1:30 p. m. the patient appeared cyanotic, respiration was quickened and the pulse was

very rapid and compressible. Large moist râles were heard over both lungs but especially over the left. In spite of stimulation, temperature, pulse and respirations rose, and the patient became progressively weaker and died at 8 p. m.

The *Clinical Diagnosis* was erysipelas.

#### AUTOPSY PROTOCOL (M. C.).

The body was that of a slenderly built, fairly well nourished white man apparently about 50 years old. Rigor mortis and posterior lividity were present. The face, neck and scalp were tremendously swollen. The eyelids were swollen shut and the surface of each lid presented a superficial excoriation which extended along the margin. The pupils were equal, there was a beginning cataract in each eye and a well marked arcus senilis. All the tissues in the region of the face and neck felt boardlike, the skin was excoriated and, especially over the anterior surface of the neck, the superficial layer of epidermis was entirely missing. There was a diffuse reddish-purple discoloration over the entire face and neck. This boardlike thickening and discoloration involved the mouth on the right side. The teeth were almost all gone and those which remained were carious and necrotic. The gums were in a fair condition. The appendix was *in situ*, lying free directly behind the cecum. There was nothing unusual about the arrangement of the intestines and no evidence of any inflammatory process in the peritoneal cavity. The omentum extended like an apron across the upper abdomen.

When the thorax was removed, the lungs did not collapse. Both pleural cavities, more particularly the left, were almost completely obliterated by dense fibrous adhesions everywhere. In the pericardial sac there was about 50 c.c. of a clear, straw-colored fluid.

When the calvarium was removed, the dura was not unusually adherent. There was an increase in cerebrospinal fluid which, however, was clear. The surface of the brain was very edematous and the vessels were all congested and engorged. The vessels at the base of the brain were markedly sclerosed. There were some old adhesions between the upper lobe and the diaphragm.

The heart was slightly larger than normal and dilated on the right side. The mitral valve, except for a few areas of fatty degeneration at the base, was apparently competent and healthy. The tricuspid and pulmonary valves were apparently healthy. There were a few calcareous plaques in the sinuses of Valsalva near the base of the aortic valve, but the leaflets were thin and the valve apparently competent. There was a well marked fibrosis of the papillary muscles and of the mural myocardium. The aorta was remarkably healthy for the age of the individual and was marked only here and there by areas of fatty change.

The right lung was rather voluminous and felt boggy although it crepitated in its entirety. There were the tags of many old adhesions throughout the pleura and beneath the pleura were numerous shotlike areas which evidently represented calcareous obsolescent tubercles. The cut surface was moist and in the dependent portions bright red and congested. There were no areas of consolidation. The left lung was almost completely destroyed in removing it. Throughout the substance of the organ one could feel very numerous obsolescent tubercles. Otherwise the tissue was merely edematous and congested.

The liver was of fair size and felt rather firm. There were old adhesions between the diaphragm and the upper surface of the right lobe which was scarred. The entire capsule was diffusely marked with fibrotic lines. There was a groove running longitudinally in the middle of the ventral surface of the right lobe, which was 2 cm. deep and 9 cm. long. On cross section, the organ appeared cloudy and was only slightly congested. The gall bladder contained about 20 c.c. of rather sticky, brownish-yellow mucoid bile, and no calculi. The mucosa was apparently thickened.

The right kidney was about normal in size. The capsule split in removing and when finally removed left a surface which was more or less torn and roughened. The organ was very firm to the feel. On cross section, the cortex was somewhat thinner than normal. The glomeruli and interlobular vessels were slightly congested and there was a diffuse fibrosis throughout the entire organ. The left kidney was slightly larger than the right. There were several retracted areas which were purple in color upon the outer surface of the cortex, otherwise the organ resembled the right.

The spleen was small, slate color, and on section showed nothing abnormal.

There were no noticeable lesions in the other organs.

*Anatomic Diagnosis.*—Diffuse cellulitis of the head and neck (erysipelas); Edema and congestion of the brain; Hypertrophy and dilatation of the heart; Fibrosis of the myocardium; Chronic diffuse nephritis (arteriosclerotic type); Peripheral arteriosclerosis; Pleural adhesions; Obsolescent pulmonary tuberculosis; Edema and congestion of the lungs.

In this case we have an example of an infection severe in degree and rapidly becoming septicemic. In this case, as in the previous one, the starting point was not discovered, and, in fact, it not infrequently happens in erysipelas that the portal of infection remains hidden. It is possible that the disease originated from the teeth, or, it may be, that it came from one of the nasal cavities. A general discussion of the origin of this disease has been given by Holmes.<sup>1</sup> For the rest, it is remarkable how little visible general organic damage is caused in these cases. Certainly the heart was poisoned—the dilatation is evidence of that, but aside from a rather moderate edema there was little change in the other tissue which could be attached to the streptococcic infection.

In a sense this case represents also one of terminal infection in an individual weakened by alcohol. It is in such cases that erysipelas is most fatal.

### CASE III.

This case exemplifies the occasional fate of the body in staphylococcus infections.

### CLINICAL NOTES.

A. G., a white boy, 10 years old, was admitted to the Cincinnati General Hospital on July 2, 1916. He died the following day. The following meager history was obtained:

At the age of 3 years he had had "hip joint disease," by which was meant that one of his legs appeared short and painful, and produced a limp. This lasted one month. At the age of 9, he had measles. During the past year he was struck in the abdomen by a swing, since which accident he has complained of burning pain on urination. Also since he was ten years old he was struck by a motor cycle, receiving a slight scalp wound. He has a history of being awkward, of stumbling over and bumping into things.

Six days before admission while at the playground, he was pushed into the water. He lit on his feet. The following day he was somewhat lame. The next day the pain in the hip was severe and the leg was swollen. Two days later he was delirious, so his people said, and a physician was called. During that night and the next day the thigh became reddened and superficial vesicles appeared over it.

When he was admitted he had not urinated for 24 hours and his bowels had not moved for four days. No other data were given in the clinical history.

### AUTOPSY PROTOCOL (J. S.).

The body was that of a fairly well developed, fairly well nourished, white boy, apparently 14 years of age. Postmortem rigidity was present in the legs but was passing away in the arms. Lividity was well marked over the dependent portions of the back. At the outer angle of the right eye and beneath the right eye, there was some subcutaneous extravasated blood. The pupils were equal and neither dilated nor contracted. On the whole anterior surface of the right thigh and the left elbow, there were some small ecchymotic areas. Over the right elbow there was a small superficial abrasion. The right leg from Poupert's ligament to about 2 inches above the knee was larger than the left and on the external aspect of the thigh, 2 inches below the anterior superior spine, were numerous (about 5) vesicles containing a clear fluid. The abdomen was slightly distended. There was very little subcutaneous fat but the muscles were well developed.

When the sternum was removed, the lungs partially collapsed. There was no excess of pleural fluid. The pericardial fluid was slightly increased, was cloudy and straw-colored and contained small floccules of fibrin. The intestines were distended with gas. The appendix was *in situ*. The peritoneum was smooth. The mesenteric glands were slightly enlarged to about the size of a bean.

On the outer lateral aspect of the right lung, there was a thin sero-fibrinous exudate, slightly adherent. On the corresponding area on the parietal pleura there was a similar exudate. Over the lateral border of the upper lobe there was a small area which did not

<sup>1</sup>Holmes: cf. MacCallum, *Textbook of Pathology*, Phila., 1916, p. 492.

crepitate and in which there was a small abscess. The tissues surrounding this area were deeply congested. The lower border of the lower lobe was deeply congested, and small pieces sank in water. The upper lobe showed, near the apex, another small abscess the surface of which was covered by a fibrinous exudate. Except for these areas, the lung crepitated throughout and showed merely congestion. The left lung had no fibrinous pleuritic exudate and crepitated throughout except for two or three small shotty areas which on section proved to be obsolescent tubercles. The apex was scarred.

The heart was of normal size and contained upon its surface a fine granular sero-fibrinous exudate which was slightly adherent and when removed left a granular surface. This exudate was equally distributed over the right heart. The heart was firmly contracted and the various cavities contained mixed clots. All of the valves were healthy. The myocardium was pale but showed no abnormality. The foramen ovale was covered by a thin membrane which could be pushed aside leaving an opening about the size of a lead pencil.

The liver was of normal size, cut easily, the edges turning out. The organ was of a deep red purplish color with mottled yellowish areas. The parenchyma was somewhat congested and showed areas of fatty change.

The spleen was of normal size, its surface perfectly smooth, it cut easily and the cut surface showed well marked Malpighian corpuscles and some slight increase in fibrous tissue.

The kidneys were of normal size, pale in color, cut easily and showed pale surfaces. The cortices were well marked off from the medulla. The glomeruli showed as fine glistening points. Throughout the parenchyma there were scattered fine abscesses varying in size from a pinpoint to 2 mm. in diameter. The capsule stripped with ease, showing deeply congested stellate veins and numerous small subcapsular abscesses.

The brain was deeply congested but not edematous and was preserved in formalin for further section.

The entire gastrointestinal tract was apparently healthy except for a slight congestion in the stomach and duodenum with a few pinpoint submucous hemorrhages near the pylorus.

On section, the medullary cavity of the right femur was deeply congested and inflamed. The process had not as yet gone on to pus formation. The hip joint contained a small amount of thin purulent material.

*Anatomic Diagnosis.*—Acute osteomyelitis; Acute purulent arthritis; Acute septicopyemia; Acute pulmonary and renal abscesses; Acute fibrinous pleuritis and pericarditis; Cloudy swelling and congestion of the liver; Congestion of the brain; Acute cellulitis of the right thigh; Multiple contusions and abrasions.

In this case a trauma, not of a severe nature, furnished the starting point for the localization of staphylococci which were floating in the blood stream. Evidently these organisms attacked the joint and the medulla of the bone, and, being of a virulent type, rapidly spread to the periarticular and periosteal tissues and produced a well marked cellulitis. At the same time they invaded the blood stream in large numbers and in transit through the organs of the body, many of them became lodged, especially in the lungs and kidneys where they produced typical focal lesions—metastatic abscesses, evidence of septicopyemia, and in the pericardium where they set up an acute inflammation.

#### CASE IV.

This case illustrates the somewhat slow progress of a serious infection terminating in septicemia.

#### CLINICAL NOTES.

B. T., a negress of 30 years, was admitted to the Cincinnati General Hospital on June 4, 1916. She died six days later. The following history was elicited: Three weeks before admission the patient missed her menstrual period. Four days later she took 10 grams of quinine and a glass of whisky, and then, a day or two later, the flow commenced. During the following five days she passed a few blood clots. Since that time she has had no bleeding, but has had a yellowish discharge from the uterus. Soon after the menstrual flow was re-established she had chills and fever, and her feeling of illness

kept her more or less confined to her bed. About a week before admission she developed pain in both sides of the neck and in the left posterior occipital region, and at that time she also complained of pain "all around the inside of her head." During the three weeks previous to admission she vomited frequently. The day before admission she was attacked by lachrymation in the left eye. The conjunctiva rapidly became bloodshot and before noon the vision was dim. This was accompanied by severe pain.

Previous to this illness the patient had been well. She was married in 1910, and had one child living and well. Her husband was well. She denied venereal disease, but for five years had had a leucorrhœa.

On admission her temperature was 104.8, pulse 104, respirations 36. She was weak, fairly well developed, and not extremely toxic in appearance. The right eye was rather dull, but the pupil reacted to light and during accommodation. The left cornea was bulging, covered with pus and was opaque. The conjunctiva was filled with pus and was congested. The ears and neck showed nothing unusual; the cervical glands were palpable. The tongue was coated, the papillæ slightly enlarged, the breath foul, and there were petechial hemorrhages in the mucosa. The teeth were in bad condition and the gums were pyorrhœic. The tonsils were small.

The thorax was well developed, and resonance and expansion were good. In the left lung there were a few high-pitched râles on expiration.

Examination of the heart showed an even rhythm, but fast rate, and everywhere systolic and diastolic murmurs were heard, with a suggestion of a friction rub. The pulses were equal and regular, but not full.

Nothing noteworthy was discovered in examining the abdomen.

Pelvic examination showed a multiparous perineum. The uterus was enlarged to the size of one at the second month of pregnancy. It was retroverted one degree, and was difficult to outline. The cervix was torn bilaterally, and was somewhat softened. The os was open. The vaults were free and not tender. There was a considerable cheesy discharge from the uterus.

The urine was clear, tea-colored; specific gravity 1005; acid in reaction; no sugar or albumin; a few hyaline and many granular casts; and some pus cells.

On the day following admission, the uterus was cleaned out. The cervix was found closed and in normal position. After dilatation some tissue the size of a prune was removed. The uterus was washed out with sterile water and with sterilizing solution and then packed with iodoform gauze. The tissue from the uterus proved to be almost completely necrotic material with a few remnants of chorionic villi. The blood taken from a vein on the day of the operation gave a negative Widal but gave a pure culture of streptococcus.

The following day the packing was removed and the uterus was irrigated. At this time the patient was very nervous and weak and had a temperature of 105 F. On the next day the right eye showed evidence of ophthalmitis, and the general condition of the patient remained as on the previous day. The next day Cheyne-Stokes breathing was present, and two days later the patient died in coma.

*Clinical Diagnosis.*—Self-induced abortion; General sepsis; Panophthalmitis (left); Sympathetic conjunctivitis (right); Pelvic peritonitis.

#### AUTOPSY PROTOCOL (L. M.).

The body was that of a well nourished, brown-skinned negress about the age of 30. Neither postmortem rigidity nor lividity was present. The pupil of the right eye was dilated. There was evidence of an infection in the deeper structures of the left eye. The cornea was totally destroyed, the eyeball atrophied and there was a slight congestion of the scleral conjunctiva.

The subcutaneous fat was much increased and the skeletal muscles were well developed. On opening the chest, the lungs were somewhat collapsed and there was no free fluid in either pleural sac. The pericardium was free and contained no abnormal amount of fluid. The omentum contained a fair amount of fat, and was adherent by fresh fibrinous adhesions to the distended intestines. Throughout the entire abdomen, the intestines were adherent to the surrounding structures and to each other by fresh, fibrinous adhesions. The spleen was adherent in like manner to the under surface of the diaphragm, as was also the liver. In the cul-de-sac of Douglas, there was an abscess, walled off, containing a greenish-yellow pus.

The right lung, of a bluish-gray color, was free from adhesions and crepitated through-

out. The cut surface was rather pale in appearance, and appeared healthy except for a moderate congestion in the base. The left lung was similar to the right.

The heart was of normal size and surrounded by an increased amount of pericardial fat. The right side was slightly dilated. On one of the leaflets of the mitral valve, there was formed a vegetation and ulceration such that the leaflet was almost destroyed. The remaining valves showed nothing abnormal. The myocardium was pale in appearance, but there was no gross pathological change. The coronary orifices were patent, and the coronaries appeared healthy, as did also the aorta.

The liver was slightly larger than normal, of a reddish hue, and fairly firm in consistence. The organ cut with slightly lessened resistance, and had a cloudy grayish appearance. There was no evident increase in fibrous tissue.

The spleen was about twice its normal size, rather soft to the feel, and on its upper surface, beneath the capsule, was a roughly triangular, reddish-blue area, about 2 cm. along its base and 1 cm. along the perpendicular. There were three other such areas scattered beneath the capsule. On section these were found to be in the substance of the spleen, and to be of the same consistence throughout. The cut surface of the spleen was a dark purple color, and very pulpy throughout. The Malpighian bodies were not visible.

The right kidney was of about normal size, and surrounded with a slight increase of perirenal fat. On the outer surface there was an area somewhat similar to those seen in the spleen, and extending likewise into the substance of the kidney. The capsule stripped with fair ease, the surface beneath being smooth and the stellate veins very slightly congested. The cortex was somewhat thicker than usual, of a grayish red color, and the line of demarcation between medulla and cortex was rather indistinct. The glomeruli were not visible, and the pyramids were rather indistinct in outline. The left kidney resembled the right, except that no infarcts were present.

The right tube was increased in diameter and its surface was congested. On opening the tube, it was found to contain a slight amount of greenish-yellow pus, similar to that contained in the abscess in the cul-de-sac of Douglas. The left tube showed a slight amount of congestion, but was otherwise apparently healthy.

The uterus was slightly larger than normal, and rather decreased in firmness. On section, the endometrium was congested and easily removed from the myometrium, the latter being rather paler than usual.

Other than the evidences of recent adhesions to the surface, and a slight congestion of the mucous membrane, the intestines showed no pathologic changes.

Cultures from the eye gave pure culture of streptococci.

*Anatomic Diagnosis.*—Generalized fibrinous peritonitis; Pelvic abscess; Purulent salpingitis; Endometritis; Acute ulcerative choroido-retinitis; Acute ulcerative endocarditis; Renal and splenic infarcts; Acute splenic tumor; Acute parenchymatous nephritis; Septicemia.

As compared with the case of erysipelas this was a chronic case. Also it was chronic in that it permitted not only the appearance of a septicemia, but also in that time was given for the cocci to establish themselves in other than the primarily infected organ, and to produce destructive lesions. Such lesions were those in the left eye, and in the heart. From the ulcerative valvular lesion emboli were carried to the spleen and kidney and there produced infarcts, which, had the patient lived long, would certainly have formed abscesses.

The thing which these case histories demonstrates best perhaps, is that comparatively slight injuries, as in the case of the boy in Case III, or a mere "lessening of resistance" as in Cases I and II, may make the proper conditions for invasion of the body by organisms which are lying in wait in the skin or, more frequently, within one or another cavity of the body. In each of the four cases, it is entirely possible that the organisms were present within the body awaiting for the proper conditions to be developed to suit their needs. They also demonstrate what serious lesions may be produced by such frequent bacterial inhabitants of the body. One may imagine that in each case the infection might have been somewhat less severe, and that the patients might have

lived. He will then suggest to himself that in the first case the man would have had a somewhat weakened heart, possibly carnified patches in his lungs; possibly peritoneal, pleural and pericardial adhesions,—(in the splenic and hepatic capsular changes there are evidences of an older infection). In the second case he might consider that the patient would become entirely well again save for a slight myocardial defect and a tendency to recurrence of the erysipelas. In the third case, the infection might result in merely a stiff hip joint, by the formation of adhesions; or in a chronic osteomyelitis with all its associations. In the fourth case, apparent recovery would be attended by localization of the pelvic abscess, and encystment of the tubal exudates, not to mention pelvic adhesions between bowel and the pelvic organs. All of these slight ailments are more frequently seen than the severe ones described in the foregoing protocols. Nevertheless both are due to the action of the same organisms the effects of which in the body depend upon the opportunities for growth, by which one means the state of the tissues with respect to their powers of resistance to infection.

# LABORATORY METHODS

## CLINICAL METHODS FOR DETERMINING THE BUFFER ACTION OF THE BLOOD\*

BY J. J. R. MACLEOD, M.B., CLEVELAND, OHIO.

COMPARISON of the H-ion concentration of normal blood with that of patients in whom for one reason or another an increase of this value might be expected (e.g., high excretion in the urine, presence of "acetone bodies" in urine, the development of symptoms usually attributed to "acidosis") has shown either no change at all or one of trivial magnitude. It has been in grave cases of acidosis alone that definite increase in H-ion concentration has been found present, thus indicating that any distinct change from the normal is incompatible with life. That this is true even when very considerable quantities of acid are being discharged into the blood, as a result of faulty metabolic processes, (e.g., in diabetes) indicates that some mechanism must exist in the blood for taking up the excess of acid without permitting any or only a trivial change in H-ion concentration. Thanks mainly to the work of L. J. Henderson, we now know that this mechanism consists essentially in the ability of phosphates and carbonates each to exist as two types of salts, acid and neutral. When these salts undergo dissociation and hydration, they do so in such a way as to yield a mixture of intermediate salts with different dissociation constants and thus capable of liberating varying quantities of H and OH ions.

Thus, let us compare the effect of adding some mineral acid to water and to a watery solution of the two phosphates ( $\text{Na}_2\text{HPO}_4$  and  $\text{NaH}_2\text{PO}_4$ ) in such proportion that it is exactly neutral to such an indicator as methyl red; whereas a drop of acid suffices in the case of water to produce a marked change in tint, it will be found that considerable quantities of acid have to be added to the solution of phosphates to produce the same change. The presence of the phosphates endows the solution with the power, as it were, of soaking up the excess of H-ions; it acts as a sponge or, less strictly, as a "buffer." In the case of blood it is mainly the salts of carbonic acid and partly the amphoteric property of proteins rather than the salts of phosphoric acid that furnish the buffer.

The above described *chemical* reactions serve to adjust the H-ion concentration temporarily, but when the addition of excess of acid is more persistent, a *physiological* mechanism also comes into play, namely, increased excretion of acid from the body, the volatile acid ( $\text{CO}_2$ ) being excreted by way of the lungs, and the fixed acid, by the kidneys.

These general considerations indicate the importance of determining the buffer action of the blood, and recently two papers have been published which seem to offer simple methods by which this can be done.

\*The manuscript for the present article was prepared some months ago, its publication being delayed through error.



## THE METHOD OF LEVY AND ROWNTREE.

A test tube made of hard ("nonsol") glass of about 20 c.c. capacity containing about a gram of powdered *neutral* potassium oxalate is filled with newly drawn blood, immediately stoppered and placed on ice. Quantities of 2 c.c. each of the blood are then placed in a series of seven small (nonsol) test tubes and allowed to stand for 5-6 minutes in order to permit a narrow layer of plasma to separate on the surface (this prevents laking of the blood during the subsequent addition of acid or alkali). The blood in the first tube is used for the determination of the normal H-ion. In each of the next three tubes are added respectively 0.1, 0.2 and 0.3 c.c. N/50 HCl, and to the last three, similar quantities of N/50 NaOH. After inverting the tubes so as to mix the contents, the blood in each is transferred to celloidin sacs and the H-ion concentration determined according to the method already described in this Journal (1915, i, p. 194).

The tubes are noted in which a change in tint from that of the normal blood is evident, and the results are expressed as the c.c. of N/50 HCl or NaOH which must be added to blood to change its H-ion concentration. Thus, the *alkali buffer* is the c.c. of N/50 NaOH which can be added to 2 c.c. of blood without change of the H-ion concentration of the dialysate, and the *acid buffer* the c.c. of N/50 HCl.

The method suffers from the following drawbacks:

1. Such small quantities of acid and alkali are employed.
2. It is often difficult to tell just exactly when a slight difference in tint has been produced.
3. Even with the precautions described above, it is impossible to be sure that the amount of  $\text{CO}_2$  in the different samples of blood is the same, which means, of course, that some bloods will, on this account alone, be able to bind more alkali than others.

A method based on somewhat the same principle, but which seems to the present writer to be more likely to be of practical value because it meets the above objections, is that suggested by Van Slyke, Stillman and Cullen. Plasma is freed of  $\text{CO}_2$  by placing it in a vacuum, and is then mixed with an equal volume of N/50 HCl (or NaOH) and the H-ion concentration determined (this can best be done by the dialysis—sulphonephthalein method of Rowntree, etc.). In the case of normal blood, after such an addition of acid, a practically normal H-ion concentration will be found, whereas in the blood of cases of acidosis it will be found to be very distinctly increased (i.e.,  $\text{P}_\text{H}$  lower). The details of this method have not as yet been published, and its principle is described here merely to indicate along what lines progress is likely to be made in the solution of this important problem.

The last mentioned workers have suggested another method, the principle of which is based on the power of the blood to take up carbon dioxide gas. Obviously blood with its reserve of alkali already depleted, as a result of the production of acid substance in the body, will be capable of taking up less  $\text{CO}_2$  than normal blood. The apparatus required for this estimation is purchasable\* and it is used in the following manner (see illustration†). Blood is centrifuged

\*Eimer and Amend, New York, and Arthur H. Thomas Company, Philadelphia.

†The originator of this method has not as yet published full details, but since the apparatus is on the market, it has been deemed advisable to give a general description of its use in this Journal.

and a few c.c. of the plasma shaken in a flask containing 6 per cent  $\text{CO}_2$ . Alveolar air is suitable for this purpose. The apparatus is meanwhile filled to the top of the graduated tube with mercury by raising the mercury reservoir F, care being taken that D and E are also filled. One c.c. of the  $\text{CO}_2$ -saturated plasma is then delivered into A and the stopcock I turned so that by cautiously lowering the level of the reservoir F, the plasma runs into B (but no trace of air).

The same procedure is repeated with 1 c.c. water, so as to wash in all of the plasma, and finally 0.5 c.c. normal acid (approximately 5%  $\text{H}_2\text{SO}_4$ ) is sucked in, after which stopcock I is turned off. The reservoir F is then lowered sufficiently to allow all of the mercury, but none of the blood, to run out of B and C.

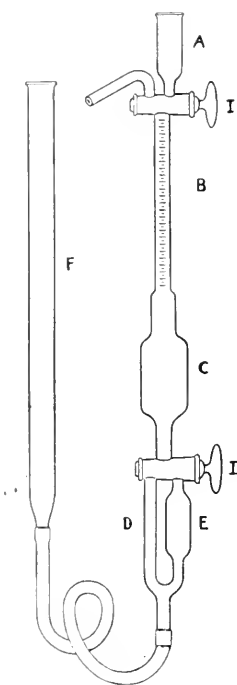
As the level of the mercury falls in B and C, the plasma effervesces violently, because it is now exposed to a vacuum. To be certain that all traces of  $\text{CO}_2$  have been dislodged from the solution, the apparatus is shaken. To ascertain how much  $\text{CO}_2$  has been liberated, stopcock II is now turned so as to bring C and E into communication, and by cautiously lowering the reservoir the fluid in C is allowed to run into the bulb E. Stopcock II is thereafter turned so as to connect C and D, and the reservoir raised so that the mercury runs into C as far as the  $\text{CO}_2$ , which has collected in the burette will permit it to go. After bringing the level of the mercury in F to correspond to that in the burette, the graduation at which this stands is read. It gives the c.c. of  $\text{CO}_2$  liberated from the plasma. Under the above conditions normal plasma binds 75 per cent of its volume of  $\text{CO}_2$ ; therefore, since the total capacity of the pipette is 50 c.c., the mercury should stand at 0.375 c.c. on the burette. In acidosis

figures as low as 20 per cent of the normal may be obtained, i.e., 0.1 on the burette.

Finally, to prevent confusion, it should be pointed out that a depression in the reserve alkalinity of the blood can also be determined by determining the percentage of  $\text{CO}_2$  in alveolar air by the clinical method already described in this Journal (1916, i, p. 522).

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## THE PURITY OF ALCOHOL

BY VICTOR C. VAUGHAN, M.D., ANN ARBOR, MICH.

IN my work with the protein poison, I have found it necessary to give attention to the purity of the alcohol used and I have found the alcohol imported from Germany (Kahlbaum's) not always up to the standard. In fact, I have found it cheaper and safer to take the ordinary alcohol and distill it with quick lime. The following tests may be used in determining the purity of alcohol:

1. The specific gravity is determined by the pycnometer.
2. It should be miscible in all proportions without cloudiness with water, ether and chloroform.
3. It should not redden litmus even after four hours' exposure. Kahlbaum's alcohol often reddens litmus in a much shorter time.
4. The evaporation of 50 c.c. should leave no weighable residue. Some samples of Kahlbaum's alcohol gave in this amount as much as 1 mg. and some more.
5. A mixture of 10 c.c. of the alcohol, 5 c.c. of water and 1 c.c. of glycerine, allowed to evaporate spontaneously on clean blotting paper, leave no foreign odor when the last trace of the alcohol has disappeared. This is a U. S. P. test and shows the absence of more than a trace of fusel oil.
6. A mixture of 10 c.c. of the alcohol and 0.2 c.c. of two per cent KOH solution is evaporated to 1 c.c. and then treated with an excess of dilute (1:4) sulphuric acid. This should not develop the odor of fusel oil.
7. 10 c.c. of the alcohol is evaporated to 2 c.c. and this is shaken with an equal volume of sulphuric acid. The development of a reddish color shows the presence of amylic alcohol.
8. When 20 c.c. of the alcohol is shaken in a clean glass stoppered bottle with 1 c.c. of silver nitrate, test solution, the mixture should not become more than faintly opalescent or acquire more than a faint brownish tint when exposed to diffuse daylight for six hours. This is a U. S. P. test for organic impurities, amylic alcohol, aldehyde, etc. The imported alcohols are not up to standard at all times by this test.
9. Into a test tube which has been rinsed with the alcohol, pour 5 c.c. of sulphuric acid, then layer the acid with an equal amount of the alcohol. The appearance of a red zone after standing for four hours or longer indicates the presence of molasses alcohol. Neither domestic nor foreign alcohols respond to this test.
10. Pass hydrogen sulphide gas through 20 c.c. of the alcohol for from two to five minutes, then add a few drops of ammonia and allow to stand for four hours. The alcohol should remain colorless. If it becomes yellowish or brownish, the presence of traces of metal, extractives or tannin is indicated. By this test many of the samples of imported alcohol are shown not to be up to standard.
11. To 10 c.c. of the alcohol add 1 c.c. of a solution of potassium permanganate (1-1000). Allow to stand twenty minutes, the development of a yellowish or brownish color indicates the presence of aldehyde. Many of the im-

ported alcohols show this impurity in more than traces. Our redistilled alcohol does not show it.

12. To 5 c.c. of the alcohol add two drops of a one per cent aqueous solution of furfurol, underlay this, kept cold in a stream of water, with 5 c.c. of sulphuric acid. The formation of a colored zone, gradually becoming pink, shows the presence of amylic alcohol. This is an exceedingly delicate test and by making a standard solution of amylic alcohol the proportion can be approximated. Kahlbaum's alcohol, as we have found, often shows appreciable traces of amylic alcohol by this test.

13. To 10 c.c. of the alcohol add ten drops of colorless analin, then three drops of hydrochloric acid. No coloration should develop within five minutes. This is a test for furfurol, which we have not found in either domestic or imported alcohols.

14. Evaporate 100 c.c. of the alcohol to dryness, extract the residue with from 3 c.c. to 5 c.c. of salt solution and inject this intravenously into a guinea-pig of from 200 g. to 300 g. weight. With our redistilled alcohol there is no effect, while with Kahlbaum's the animal is often thrown into convulsions which may terminate fatally. What this residue contains, I have not been able to determine. It is granular and sometimes contains a few needle shaped crystals.

From my experience, I have found it better to use alcohol prepared in my own laboratory from the commercial ninety-five per cent article, by redistillation with caustic lime, than to depend upon the imported product.

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## WATER BATH SLIDE METHODS FOR COMPLEMENT FIXATION AND BLOOD GROUPING\*

BY NORMAN E. WILLIAMSON, M.D., STOCKTON, CALIF.

A COMPLEMENT fixation test may be performed on a slide with the small quantity of material according to the following technic:

A rectangular water bath is fitted with thermoregulator at one end. Lateral flanges are placed  $\frac{1}{2}$  inch from the top. The bath is filled to within 1 inch of the flanges. On this ledge rest the ends of two glass rods placed across the water bath 2 inches apart. To the glass rods so that it is below them but not long enough to rest on the ledges is tied a rectangular piece of cardboard. There is a narrow slit in this cardboard lengthwise, half way between the rods. The object of this is to maintain the proper amount of moisture on the under side of a slide placed across the rods. If there were no cardboard, too much moisture would be precipitated on the slide and hemolysis of a drop of blood might occur from dilution. If the cardboard is not perforated or slit, the under side of the slide will dry. The size of the slit must be such that a faint haze forms on the under side of the slide which promptly disappears after the slide is removed from the water bath. Over the top of the bath is placed a glass plate except for the end occupied by the heat-regulating apparatus. The open space here is

\*From the State Hospital, Stockton, Calif.

closed by a vertical glass plate setting into the water but allowing free interchange of water below it. The plate of glass on top of the bath must not fit too snugly or there will be a loss in a short time of atmospheric dust, and moisture will be precipitated on all objects in the bath. Blotting paper is attached by adhesive to the under side of the glass plate to prevent dripping on the slides. The thermometer is placed on the rods and the heat-regulating apparatus so arranged that the temperature at that point will be  $37^{\circ}$  C. Constant level is maintained by a filled inverted water bottle with the mouth placed at the level desired.

For the performance of the test very small quantities of standard reagents are prepared. Clean slides, 3 small standard platinum loops, 1 large platinum loop and one straight platinum wire are necessary. The slides are placed on a sheet of paper, each on a number. A loopful of material is taken for the smallest quantity of any of the ingredients of the test and larger quantities of other ingredients are multiples of this smallest amount. For instance, in performing Noguchi's modification of the Wassermann test, it is done as follows: 1 small loop of Noguchi antigen properly diluted is placed on the slide as shown (Fig. 1); 1 small loop of undiluted complement; 2 small loops of patient's serum; 1 large loop of salt solution. These are then mixed with the straight wire and quickly inverted over the water of the water bath across the rods. The time is marked on the paper by the number. Incubate  $\frac{1}{2}$  hour at  $37^{\circ}$  C.

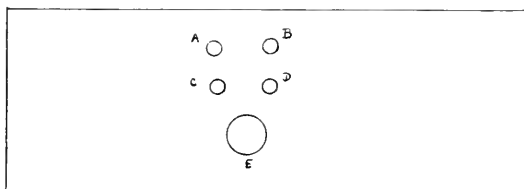


Fig. 1.—A, complement; B, antigen; C, patient's serum; D, patient's serum; E, salt solution.

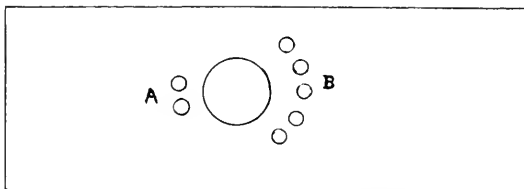


Fig. 2.—A, amboceptor; B, sheep's cells.

Remove slide and add 5 small loops of 10 per cent suspension of sheep's red corpuscles and 2 small loops of amboceptor which is of such a strength that 5 units are contained in 0.5 c.c. (Fig. 2). Mix with a straight platinum wire and replace in water bath. Incubate for 1 hour. Positive specimens are brick red and opaque; negative are clear and brown.

The strength of the complement has been previously determined as follows: One loopful of complement is added to each of 4 loops, 3 loops, 2 loops and 1 loop of salt solution, and one loop of each mixture and also of the undiluted complement is added to 5 loops of the 10 per cent suspension of sheep's corpuscles and 1 loop of amboceptor in the strength given above, and placed in the

water bath for one hour for hemolysis. Twice the strength of the smallest amount of complement giving complete hemolysis is taken for the test.

For Noguchi's test use 2 loops of serum and one loop of complement in case the strength of the complement is such that one unit is 5/100 of a c.c.; 1 loop of Noguchi antigen which has been prepared by adding one part of the methyl alcoholic solution to 7 parts of salt solution; 5 loops of 5 per cent suspension of washed human corpuscles and 2 loops of amboceptor which has been prepared in the ratio of 5 units to 0.2 of a c.c. of salt solution.

Each specimen should be duplicated without antigen to determine anticomplementary action of the serum. The usual positive and negative controls are required. A positive result as above equals ++. If 1 loop of serum gives a positive result it would be ++++. Find the smallest quantity of serum which will give complete inhibition and rate accordingly. By this method the presence of antishoop amboceptor in human blood may be disregarded as excess of amboceptor will have no effect if all the complement is fixed. The same rule is applicable in the use of the human hemolytic system.

Two loops full of serum can be dried on a slide and later dissolved with salt solution and used for the complement fixation test. I have found that the dried blood may require more complement than the fresh blood and I obtained the best results by using sensitized red cells. For this purpose it is well to have an amboceptor which contains comparatively weak agglutinins. To the washed cells in a large quantity of salt solution was added enough amboceptor to make 3 units for the entire quantity of cells. The mixture is kept at 37° for two hours or left in the ice box over night. The cells are then thrown down, the fluid decanted, and fresh salt solution added to make the proper dilution. The complement is titrated with sensitized cells and the best results with dry blood are obtained if twice the amount of complement which will completely hemolyze these cells in 15 minutes, is used. Known positive and negative dry sera must be used for controls.

Kaplan adds his amboceptor to his cells prior to the performance of a Wassermann reaction and his cells are more or less sensitized according to the time and temperature, but he does not make a special effort to have them entirely sensitized as is done in this test. There seems to be no reason why dry blood could not be used for the test tube reaction. 1.10 of a c.c. of serum weighs when dry 10 milligrams; thus 20 milligrams of dry serum could be added to each tube for the Wassermann test after dissolving in salt solution and 8 milligrams for the Noguchi test. Serum can be dried on a clean piece of glass, scraped off, put in a homeopathic phial and sent to the laboratory. It need not be sterile. I have dried some by this method and will test it in a few days.

Since serum is usually readily obtainable fresh the water bath method would not often be required for the diagnosis of syphilis but it may have a distinct value when bacterial emulsions cannot easily be obtained for antigen in the quantity desired for the complement fixation. Test tube methods are easier to perform when control of all conditions is considered.

This water bath slide method was used by me for the purpose of determining the grouping of bloods, using the loops of material as advised by Brem but using no cover-glass or well slide. I use for the test sera of groups 2, 3

and 4 of Moss which have been found by me to be strong in agglutinins. Testing the suspension of corpuscles of the patient with strong sera of groups 2 and 3 will place his group. If the patient happens to have a weak agglutinin for his group, this method is an advantage. For instance, if the corpuscles are agglutinated by group 2, not by group 3 sera, the patient belongs to group 3. If agglutinated by group 3, not by group 2, he belongs to group 2. If agglutinated by both he belongs to group 1. If agglutinated by neither, he belongs to group 4. A further check with the group 4 serum is then used. Since the corpuscles of any group other than 4 may be affected by hemolysin in a patient's serum, it is advisable in using the human hemolytic system for complement fixation test, to use only a group 4 suspension of corpuscles. I obtain them easily as follows: After pouring the serum off the bloods obtained from the patients for Wassermann, I make a suspension of a few of the red cells which I judge to be approximately 5 per cent and see if they will be agglutinated by a group 4 serum I keep in stock. If they are not the blood belongs to group 4. I then shake out of the clot by the aid of a little salt solution some red cells, wash them in the usual manner, using them for the fixation test. I have found that on an average without getting either fibrin or leucocytes I can shake out 3/4 c.c. of red cells from my bloods by this method. I use such cells for the immunization of rabbits after having determined that the Wassermann in that case is negative and the history of the patient negative. Such cells can be mixed safely only after all the serum has been removed. I killed two rabbits by a failure to observe this precaution as agglutination of corpuscles may occur if the blood belongs to different groups. I find this much the easiest method to get normal cells.

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## *EDITORIALS*

### *Medicine as a Career\**

**S**TUDENTS of Medicine: You have chosen your life work. You have elected to devote your time and energy to the science and art of medicine. It is hoped that you fully realize the importance of this decision and that you have not come here without adequate deliberation and comprehension of the heavy tasks you have assumed in taking this step. In view of the possibility that some of you have made a mistake, I have decided to spend this hour in presenting to you a few of the duties and obligations which you are assuming, and if there be among you those who feel that the burdens to be borne are too heavy and the personal gain too light, let such not hesitate to stop and turn back on the threshold. Medicine needs recruits, but it desires and will accept only those who after severe tests, it deems worthy. I am aware of the fact that the words of the experienced fall lightly upon the ears of the inexperienced, but one who has served in the ranks for nearly forty years offers you advice. I wish to say that the fatality among medical students is great. In the past ten years, less than sixty per cent of those who have entered this school have succeeded in winning its diploma and of those who have gained this distinction, not

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\*Address at the opening of the University of Michigan Medical School, Oct. 3, 1916.



ll have fulfilled the confidence imposed in them by the faculty. It is not my expectation that you will do better than preceding classes.

Medicine embraces all facts which may be utilized in the prevention or alleviation of disease. Its chief contributory sciences are physics, chemistry and biology. It is for this reason that a knowledge of the fundamental principles and facts of these basic sciences is required for admission to the better medical schools. Some of you will fail because your training in these sciences has been inadequate. Teachers in the medical school cannot take the time nor can they hold back better trained students to instruct those who are deficient. By the end of the first year most of these unfortunates are asked to withdraw.

With the best possible preparation the medical student finds his daily task, quite as much as the strong can carry and altogether too heavy for the weakling. There has been some discussion among medical educators concerning the curriculum, some contending that it is too heavy for the average student. This depends upon what is meant by the "average student." If the standard set in college work is applied, I am of the opinion that medicine does not want such "average students." I am convinced that a strong student, of a high average, can carry the medical work as now imposed and that the imposition of a heavy task succeeds in weeding out the unfit and is therefore desirable. We do not develop muscles by lifting feather weights, nor do we strengthen brain activity without earnest effort. The aim of medical education is to develop strong men and in order to do so, difficult tasks must be imposed in the training.

A strong intellect is not enough to insure success to the medical student. Intellect must be backed by industry, otherwise it is of but little value. For lack of industry many medical students fall by the wayside. After forty years as a teacher in this school, I am of the opinion that lack of proper application to the work is the most potent cause of failure among the students. In his collegiate course the work has been light, easily done. He has had a good record, but has failed to establish habits of study. Some allurements cause him to neglect his tasks for a day and then for a week. Soon, he finds himself quite in the rear. His bluff at recitation does not go. His teachers question his intellectual strength and honesty. He becomes a derelict and must be removed for his own and others' good.

A third essential to success in medicine is integrity. When endowed with a high degree of intelligence, supported by the greatest industry but without integrity the medical man is likely to prove a disgrace to his profession and a menace to the community in which he lives. That integrity has been regarded as an essential qualification of the practitioner of medicine from the earliest times is shown by the exaction of the Hippocratic oath supposed to have been formulated by the father of the profession. The medical man must be honest with himself, his patients and the public. For personal gain he must not pretend to greater knowledge or skill than he possesses. Professional ethics insist that in the announcement of his purpose to serve the community he must restrict himself to the simplest statement. The public has long ridiculed the restrictions which the medical profession has attempted, with more or less success, to impose upon its own members, but that the public is now reaching a point where it appreciates the righteousness of medical ethics is shown by recent

legislation forbidding false and exaggerated advertisements. The first thing for the honest man in becoming a physician to do is to secure the best possible preparation. To enter upon the practice of medicine or to continue in it without adequate preparation is a crime—a moral, if not a statutory one. The public has come to this view and there is no other profession, admission to which is so strictly guarded as that of medicine. State laws set the standards of admission to medical schools and State Licensing Boards test medical graduates. The best intentions do not supply the deficiencies due to lack of knowledge and skill. It certainly can be said that in the practice of medicine, knowledge is a virtue and ignorance a crime. Recognizing the fact that no man, however great his intelligence and untiring his industry, can be skilled in all branches of the healing art, individuals select specialties in which they strive to make themselves experts and these so group themselves that each patient may have the advice of an expert. The wisdom of this procedure and its advantages to both practitioner and patient must be evident to all. Each individual in such a group must know his specialty and must keep in touch with its progress. Medicine is a progressive science. Each year adds to its effectiveness. Discoveries in physics, chemistry and biology find practical application in the prevention or cure of disease. It follows that the efficient medical man must continue to be a student so long as he remains an active member of the profession. In medicine there are no "papal bulls," no "ipse dixits" and even "precedent" is shown but scant respect. It is best compared to a living plant constantly drawing sustenance from soil and air, dropping its withered leaves and branches, ever putting forth buds and blossoms and bearing each season better fruit. One who is not capable of sustained effort, should seek some other calling in life. Occasionally I meet with men who are still living professionally in their undergraduate days, reading the same old books, and writing the same old prescriptions, both blind and deaf to the changed environment. Fortunately the more intelligent of the public easily recognize these fossils and appraise them at their true worth. They are interesting as relics of the past but worthless in the present.

From the time of Hippocrates to the present, wise men in the profession have always advocated amity among its members and I must say after many years of personal experience that there is no other high professional ideal so difficult in realization, but I am proud to add that there never has been a time when the promise of the realization of this ideal has been so great as at present. In this matter medical men have learned much from the commercial world in which the value of cooperation has been so abundantly demonstrated. The efficiency of the individual has been increased and the value of the product has been improved. Much regret has been expressed concerning what is called the passing or the elimination of the old-time family physician or general practitioner. In the slow development of scientific medicine he served his fellow-men, often with the greatest devotion and self-sacrifice. The history of epidemics shows him to have been often worthy of the highest honor. He has faithfully served his fellow-men in times of dire distress. Occasionally he has made contributions of the greatest value to science. In the record of the slow progress of man from the marshes of ignorance and superstition to the uplands of knowledge and

science he bears a conspicuous and honorable place, but in the practice of modern medicine his part is a subordinate one.

In any community in which several physicians are singly doing a general practice, cooperation, with the development into skilled specialists, results in individual efficiency among the medical men and better service to their clientele. With a properly equipped hospital at their service, a group of village physicians may give their patients the same scientific and effective treatment that they can secure in larger medical centers. I have no sympathy with the contention that our rural population demands cheaply educated physicians. With trolley cars and automobiles there are but few in need of medical aid who are so located that a good physician may not soon reach them, or what is better, that they cannot soon be transported to a hospital. A friend who has long practiced in a small Montana city recently told me that twenty and more years ago, his ride sometimes carried him one hundred and twenty-five miles from home. One such visit to a case of pneumonia is unsatisfactory to the doctor and of but little benefit to the sick man. Now, the automobile brings the patient to a well equipped hospital where several physicians may daily consult concerning the case and trained nurses be constantly in attendance.

When I was in medical practice, like the milkman, I made my daily rounds seeing cases of scarlet fever, diphtheria, sometimes smallpox, pneumonia, various nervous diseases, attending cases of labor and in short, I was a general practitioner. Like others of the kind I did the best I could for all and I am able to say with some pride that in no case did I carry infection from house to house. However, in order to avoid this I often had to change my clothing and disinfect my person many times in one day. This kind of practice is still largely in vogue, but it is gradually being displaced by hospital treatment in which specialists direct and trained nurses administer. The greatest need of practical medicine today is more and better equipped hospitals. With these the specialist and the skilled nurse will multiply and improve. Such hospitals should be supplied with thoroughly equipped and competently manned diagnostic laboratories which should serve not only curative but preventive medicine. Water and milk supplies should be examined daily and visiting nurses under the direction of a competent health officer should constantly patrol the community. Adjunct dispensaries which should serve as schools of instruction in baby feeding and care, child welfare, home sanitation and in everything pertaining to healthy living should supplement the community hospitals. When in addition to these agencies the people generally can be educated to see the benefits that would follow the periodic thorough examination of all in order to detect the first departure from the normal, then medicine will be able to render its highest service to mankind.

It must be evident that if these hopes are to materialize the practice of medicine must become more and more a State function. That the tendency is in this direction and that this should be encouraged for the public good are not matters of doubt in my mind. Only a few years ago some of the most eminent men in the profession combatted earnestly State support of medical education. They claimed that the State had no right to establish and maintain medical schools and that such aid was not fair in competition with the proprietary schools, which at that time educated more than ninety per cent of the annual recruits to the profession. Now, no one questions either the right or the duty of the State to

establish and support medical schools, while the proprietary schools, having proven wholly inadequate and inefficient, have practically ceased to exist. Even the man in the street sees the advantages that have resulted from these changes. To state that a medical school is a proprietary one, in the sense generally understood by that term, immediately condemns it with intelligent men. Courts from the lowest to the highest in the land have uniformly held that the State has the right to maintain its own medical school, also to pass upon the merits of other schools both within and without its borders, to set up standards of medical education, to define the requirements of admission to medical schools, to submit those who wish to practice within its borders to certain intellectual and moral tests in order to pass upon their professional fitness and to revoke the licenses of the unworthy. The Federal Government has its public health service which passes upon immigrants, controls national quarantine, maintains a research laboratory, supervises the manufacture and sale of vaccines and antitoxins, and stands ready to aid any State in combatting epidemics. Each State has its Board of Health, the powers, functions and efficiency of which vary widely. Our great municipalities have their boards of health and commissioners of health which for the most part are efficient, but in some instances are parts of a political machine. Our smaller cities and rural communities have their boards and health officers, which with some notable exceptions, fortunately in increasing numbers, are cheap, ignorant and inefficient.

By means of these organizations, imperfect as many of them are, the death rate in the registered area of the United States has been reduced in the past thirty years from twenty to fourteen per thousand, the average life has been increased more than ten years, and the mortality from tuberculosis and other infectious diseases has been reduced about fifty per cent. On account of the greater efficiency of the health service in our larger cities, the reduction in the death rate has been more marked in these than in smaller cities and rural communities. The greatest reduction in mortality has been secured in our cities of one hundred thousand or more. Our metropolis, New York, has a municipal health service which is second to none in the world. It supports a research laboratory in which the highest grade of scientific investigation is done, diagnostic laboratories in which diphtheria cultures, suspected sputum, blood examination and other tests essential to scientific medicine are made and laboratories in which water and food supplies are carefully guarded. It has a corps of expert diagnosticians ready to aid the practitioner in all suspected cases, free of charge to either the medical man or the patient. It provides medical school inspectors who detect infection in its earliest stages, excellent hospitals in which the sick have the best care and treatment and nurses who patrol the tenements and other homes of the poor and give instruction in sanitation. It examines cooks and waiters to see that none of these may distribute typhoid fever, tuberculosis, syphilis or other infections. It inspects meat markets, bakeries, milk stations and other places of food supply and has the authority to close these when unsanitary conditions are found.

The last legislature of Michigan made an appropriation of one hundred thousand dollars and directed the State Board of Health to expend it in attempts to restrict tuberculosis. Several thousand citizens have already been examined free of charge in order that this disease may be detected in its early stages when it is amenable to hygienic treatment. These people are not only examined, but

those found infected are instructed how to live in order to avert the progress of the disease.

I have chosen to bring these matters before you in order to impress upon you the relation which the profession, which you have selected, bears to the public. Even the physician who devotes himself wholly to what is known as private practice does not escape his duties to the public. He is morally bound not only to do his full duty to the individual, who employs him, but to protect the community. You have chosen to come to a school supported by the State. Michigan practically gives you your education. Why does it do this and what does it demand of you in return for this great gift? It expects that you possess intelligence, for without this, the gift is valueless; that you manifest industry both during and after your student life, for without this, you bury your talent; that in all your actions, both professional and nonprofessional, you show the most sincere integrity, for without this, you become a menace to your benefactor. The State has selected this faculty to ascertain to what extent each of you possesses these essential qualifications and I can assure you that those found wanting will not find their way into the profession through these doors. To those, who prove worthy, every reasonable encouragement and proper assistance will be given.

I am sometimes asked what financial reward can the medical man reasonably expect? This is a proper question and I am ready to give it my answer. In the first place a medical education, even with the relatively small tuition one pays in a State University, is the most expensive professional education, both in time and money, both to the State and to the student.

The laboratory expenses of the medical student are higher than those in any other school. Where other students buy books, he buys not only more expensive books, but he must also purchase a microscope, blood counter, and other expensive instruments. After graduation most medical students spend from one to three years in hospital work and at least one of these promises soon to become obligatory on all. When he begins practice the medical man must have a respectable office and a well equipped laboratory. He must continue to buy expensive books for the average medical book is out of date almost as soon as it leaves the press, so rapid has been the advance in scientific medicine in the past thirty years. He cannot do without the best professional journals and being a member of a learned profession he is ashamed to be ignorant of the best general literature. In his consulting room, his visits to the homes of his patient and in his association with his fellows he must be neatly, though he need not be expensively, dressed. He must supply himself with means for quick and comfortable travel. Without going into further particulars I may say that by the time he is ready to begin his professional work, the most economical medical man has already made an investment of from ten to twenty thousand dollars, counting his actual expenses, allowing a fair amount for his time and calculating the interest on these amounts, and when he begins, he must have the wherewithal to make his work successful. No medical man can neglect the financial side of his life's work. Without an adequate income he cannot reach a high degree of efficiency in his work. However, the medical man who is imbued with the right spirit will use his financial gains largely in increasing his professional efficiency. After setting aside enough for the fair support of himself and those dependent upon him, he will devote the surplus — and there must be a surplus if he is to be

successful—to better equipment, both physically and mentally. It has been my observation that the more intelligent laity respects the physician, who endeavors to keep himself well posted and well equipped in his professional work. Medical men who attend their local, State and national societies are as a rule successful financially while those who think that they cannot leave their work even for self-improvement, have a hard time in making ends meet. One who wishes to accumulate a fortune or to become wealthy as that term is now understood, should choose some other calling. I know of no one who has placed himself in this class by the reputable practice of medicine. Some medical men have made riches by fortunate investments, but this is an exception. Some marry wealth, but this is usually fatal to professional efficiency. I know of but one man who has demonstrated his ability by winning the highest distinctions in the profession notwithstanding the fact that he married a wealthy woman. While on this point, I may say that practice coming from the ultra-rich is not to be coveted. They are exacting in their demands and poor pay. They object to ordinary bills and cry out that they are being sandbagged. As I write this, I have before me such a letter from a millionaire. He admits that he selected the medical man on account of his recognized skill, that he knew what the charges would be before the services were closed and that he did not object at that time, because he was afraid that the medical man would desert him, but when payment was demanded, he claimed that he was being sandbagged because he was known to be rich. The ultra rich are familiar with the use of the sandbag in extorting money from others and they see its phantom in even the most moderate bills presented them.

The medical practitioner endowed with intelligence, fortified with industry and with his every action controlled by strict integrity is sure to make a decent living, care for himself and family in comfort and he need not sleep in a pauper's grave. He is not compelled to sacrifice his self-respect to expediency. His calling is quite as independent as any other. He can choose his own friends, church and political affiliation. The man who is sick with pneumonia, or has an inflamed appendix does not consult the society columns, the church directory nor the polling lists when he selects his medical attendant. He prefers the man who is likely to render him the best service and the intelligent public in the long run and on the whole judges wisely. There never has been a time when individual worth among medical practitioners was more correctly evaluated and I may add, more highly estimated, than the present. Medicine has cast off the veil of mystery which once covered her face and walks among men, uncovered and unashamed. The days of "divine healers," Indian medicine fakirs and of Mrs. Winslow and Lydia Pinkham are passing away. Some may say that these statements are contradicted by the wide prevalence of christian science, osteopathy and other cults. These are only the vagaries which have taken form in the delirium racked brain of a fast dying superstition. Did our Government select any of these agencies in its successful combat with yellow fever in Cuba or on the Canal Zone? Has it relied upon them to keep Asiatic cholera or the plague out of this country? Did it send christian scientists or osteopaths to stay the epidemic threatened by the Dayton floods? Are these cults now busy healing the wounds and adjusting the dislocated bones so abundant on European battlefields? Our Lady of Lourdes and Ste Anne Beauprie are apparently not on duty at a time when shell-

torn and flame-tortured humanity is in greatest need of their much extolled, miraculous powers of healing. The genuine worth of scientific medicine has never been so thoroughly tested as in the present war. Amid unprecedented difficulties, in the camps where millions are congregated, in the quick transportation of corps after corps, in the trenches and even among the prisoners of war, always cared for grudgingly and reluctantly, everywhere, preventive medicine has successfully met her old foes, typhoid fever, dysentery, cholera, tetanus, and other epidemics, which in former wars have usually been the most destructive factors in the midst of contending armies, and have often decided battles and determined the fate of nations. Decisive victories have not yet followed the flags of the central or the allied armies, but in all, the Red Cross signalizes the most triumphant achievement of man. International laws have been torn into shreds and become mere scraps of paper, moral and religious precepts and codes have been supplanted by brutalities never practiced by primitive man and the foundations of civilization have seemed to be on the point of disruption and final collapse, but the spirit and ideals of scientific medicine remain unsullied and a new world in which these shall dominate will be created.

Medicine offers a number and variety of special activities to those who choose it as a career. First, there is the grand division into preventive and curative. The former is a product of the Nineteenth Century, the latter as old as the records of man. The oldest and still a widely dominant theory, as to the cause of disease is that it is an infliction laid upon man by some supernatural being. Primitive man, which term once embraced all, and in this particular, still includes many, probably a majority, even among the most highly cultured nations, believed in the existence of powerful spirits, who measured out good and ill to individuals as their own will might indicate. The religion of such believers consisted and still consists in attempts to propitiate these powerful, or one omnipotent, spirit. They built and still build altars of sacrifice and temples of devotion in which they proclaim their own weakness and implore divine protection and guidance. They still beseech a supreme ruler to shower blessings upon themselves and curses upon their enemies. In the hands of the Jehovah of the Jews disease was a scourge for the punishment of those who merited his displeasure. In the adoption and modification of the Hebrew religion by the Christian world, the idea of a God of wrath was adopted, and still prevails. Even today in battle scarred Europe, the same God is invoked and his aid asked in each contending army. With this inborn superstition transmitted through countless generations, scientific medicine has had to contend. The combat has extended through centuries as is shown by the earliest records of human achievement. The first signal victory was won when Jenner robbed smallpox of its horrors by the discovery of vaccination and success was assured by the labors of Louis Pasteur who marked the way by which each infection may be identified, controlled and abated.

An enlightened public is beginning to recognize that many diseases, especially the infections, are preventable and the medical profession is being called upon to plan and direct this work. Many of the smaller cities and some rural communities are providing for full time health commissioners and the demand is greater than the supply. This and other universities are conducting courses specially suitable for public health officials. I am sure that some of you will

select this field for the development of your life work. In it there is abundant opportunity to do credit to yourself while you serve the highest interests of your fellow-man. The labors of Reed and his colleagues demonstrated the agencies by which yellow fever is spread and Gorgas and his helpers freed Cuba from this disease and won a greater triumph in the Canal Zone. Laveran and Ross did even a greater service in showing how the world may free itself from malaria, which in all times has held some of the fairest and most fruitful lands under its curse. Preventive medicine is now capable of opening up the tropics as suitable habitations for civilized man, of removing the stigma of being the "home and nursery of disease" from the fertile valleys of the Nile, and of returning to cultivation the banks of the Euphrates and the Tigris on which the cradle of civilization was rocked. I cannot believe that coming generations will be so insane as not to use this most potent agent in reclaiming the marsh, the wilderness and the barrens, and converting them into fields, rich in agricultural products and abundant in happy homes. Man's destiny is in his own hands and he may make of this earth a heaven of peace, plenty and prosperity, or he may mar it into a hell of strife, rapine and murder. In knowledge he has advanced to a position in which he becomes a co-worker with the Creator, and he must bear the responsibilities which such power imposes. In the struggle between good and evil, knowledge and ignorance, science and superstition, medicine has, and must continue, to lead the way, and you as its standard bearers must serve your day and generation with intelligence, industry and integrity. I do not mean that you are to do your work, always conscious of the burden of duty. With all its imperfections this life is worth living and its highest joys lie in its contests. The man who does not get real pleasure out of his work remains a poor workman and his products do not find ready sale in the market. Even the bitterest disappointment, when you have done your best, often becomes a beacon light warning you of the rocks and leading you into a safe harbor.

It must not be inferred from the great stress that I have placed upon preventive medicine that the curative art is not equally worthy. Moreover, cure is not going to be replaced wholly by prevention. Disease and accident will continue so long as man reproduces his kind. The history of this, the older, branch of medicine, is that of man's efforts to relieve the distress and to minister to the needs of his fellow man. Born in ignorance, nourished on superstition, clothed with mysterious rites and ceremonies, medicine has had a hard task to free itself from hereditary and environmental influences. Attempts to break away from these adverse and retarding conditions have marked the highest efforts of the race. During nearly every century since recorded history began, there have been some superior men, intelligent and far-seeing above the masses, who have contributed something to science. Such were Hippocrates, Galen, Pare, Servetus, Harvey and others whom we now delight to honor as contributors to knowledge and benefactors to the race. The discoveries, by empirical methods, of the specific effects of Peruvian bark in malaria and of mercury in syphilis did much to improve the condition of life and to enlarge the field of human endeavor. Since the scientific era began, the marvelous virtue of antitoxin in diphtheria; its great value in tetanus; the relief of cretinism by thyroid feeding; the action of thymol in hookworm disease; the benefit of salvarsan in the treatment of syphilis; the Pasteur treatment of hydrophobia; the prevention and cure of berri-



berri by nutritional regulation, mark some of the most evident achievements in curative medicine. For diagnostic and prognostic purposes the medicine man of primitive peoples consulted oracles, watched the peristaltic movements of the intestines of animals offered in sacrifice, or read the fate of his patient in the positions of the stars. The physician of today employs the discoveries in physics, chemistry and biology for these purposes. The physician of fifty years ago was compelled to rely largely upon the study and interpretation of symptoms in which the best became highly proficient; today he supplements these studies with the microscope, Roentgen ray, test tube, and other instruments of scientific precision. Then, his conclusions were drawn largely from guesses, now they are founded upon exact and positive knowledge. A large part of your undergraduate education will consist in familiarizing yourselves with the use and application of instruments of precision for diagnostic purposes. Each year brings forth advances in the fundamental sciences and medicine is ever ready to utilize such discoveries as may be of service in the prevention or cure of disease. It has been demonstrated that the physiological action and therapeutical effects of a chemical compound can be modified by changes in its molecular structure. The genius of Ehrlich produced salvarsan and its later substitutes in accordance with this principle and the possibility of finding curative agents in other diseases by similar investigations is now occupying the time and energy of many laboratory students. While the achievements of preventive medicine have greatly reduced the numbers of those infected, medicine is not neglecting its curative agents and we can confidently expect great results in this direction.

The advance of modern surgery has been marvelous. No greater gifts has science brought to suffering man than surgical anesthesia, the discovery of which American medicine can justly boast, and aseptic surgery, made possible by the fundamental work of Pasteur and given practical application through the genius of Lister. These discoveries enable the surgeon to penetrate every part of the body and remove diseased tissue, repair injuries, extract foreign bodies and restore the individual to health and efficiency while he sleeps wholly unconscious of the operation. Plastic surgery has become a fine art and the successful transplantation of tissue is being practiced in the base hospitals of Europe, where the brutalities of man are being ameliorated by skillful operation. The possibility of not only preserving but of growing animal tissue *in vitro* has been demonstrated and has developed a reasonable hope that the surgeon of the future may do still greater miracles.

The development of medicine must be preceded by scientific discovery, because medicine consists in the application of these discoveries. It follows that the highest duty of the medical man is to make contributions to scientific advances. In the past, medical men have made an honorable record in this direction and there is no branch of science to which they have not brought valuable contributions. Even at the present, the open field of knowledge is of small dimensions, while on every side extends the boundless wilderness of ignorance. It has been a great privilege and a joy to have lived at a time when my chosen profession has been so rapidly moving forward and to have met face to face so many of its leaders. It has been my fortunate lot to work in the laboratory of that great German, Koch, to have listened to the words of that great English-

man, Lister, to have enjoyed the friendship of that great Russian, Metchnikoff, and to have looked into the kindly face of the greatest man of the generation, if greatness be measured by good done one's race, that Frenchman, Pasteur. May some spark of the genius which led these men to great accomplishments descend upon and abide in you.

—V. C. V.

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## *ORIGINAL ARTICLES*

### A STUDY OF THE LIPIN CONTENT OF THE LIVER IN TWO CASES OF DYSPIUITARISM\*

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THE question of the fat-content of the various organs and tissues, in normal and in pathologic conditions, has been the subject of much discussion and research during the last decade. As the result of numerous investigations we know today that the majority of the tissues and all of the organs of the body contain fat under normal conditions. As regards the presence of fat normally in the hematopoietic organs there is still some conflict of opinion; but in certain pathological conditions these organs show marked accumulations of lipoid bodies showing that they must bear some important relation to certain forms or phases of fat-metabolism, at least. Great interest has been aroused by the discovery of the important part played by the lipoid bodies in various pathologic processes, and lipoid cells (cells with large amount of pale, vacuolated, foamy, reticulated cytoplasm and small, deeply staining eccentrically placed nuclei) are at the present time attracting much attention in pathologic histology. They have been found in many pathological processes; in inflammatory infiltrations, particularly lymphoid areas, in chronic aortitis, appendicitis, salpingitis, pyelitis, old ovarian abscesses, chronic mastitis, chronic cholangitis, prostatitis, chronic nephritis, actinomycotic abscesses, chronic granulation tissue, leprosy, rhinoscleroma, chorioid plexus, adrenals, neoplasms, etc. The xanthoma and pseudoxanthoma cells are lipoid cells. As to the histologic origin of the lipoid cells there has been much discussion; they are probably fibroblastic (reticulo-endothelial) cells in a state of lipoid infiltration (lipoid macrophages).

The chemical nature of the lipoid bodies represents still an almost unknown field. The school of Aschoff divides them into three groups, *glycerin-esters*, *cholesterin-esters*, and *lipoids* in a narrower sense (phosphatid, cerebrosid, fatty acids, and soaps). Mixtures of these substances, particularly of cholesterinesters

\*From the Pathological Laboratory of the University of Michigan.

and lipoids, are not rare. Histologically the lipoids may be demonstrated by staining with osmic acid, Sudan III, Scharlach R, Neutral red, Nile blue; by the methods of Smith-Dietrich, Fischler, and Ciaccio, and by polarized light. The cholesterol lipoids are doubly refractive (anisotropic) and stain less black with osmic acid, and more yellow or brownish with Sudan III and Scarlet R than do the glycerin-esters.

Aschoff regards cholesterol-ester infiltration as a sign of chronic inflammation or of a chronic disturbance of metabolism. According to his views, in conditions, such as glycosuria, pentosuria, and chronic icterus, in which cholesterolin-esters are present in abundance in the blood-plasma (cholesterinaemia) the cholesterolin-esters are taken up by the cells (anisotropic fatty infiltration). However, Aschoff's views are not generally accepted, and by some writers are regarded as premature.

Our conceptions of the lipin metabolism of the body have recently been greatly widened by the discovery that lipid bodies accumulate in the spleen and blood-forming organs in certain pathologic conditions, notably diabetic lipemia and Gaucher's disease. In 1903, Fischer noted in a case of lipemia that the spleen was hyperplastic and showed a diffuse infiltration of fat-droplets. Not until 1912 was a similar observation recorded when W. H. Schultze reported a case of diabetic lipemia in which the spleen showed almost complete replacement of the pulp with large, pale, vacuolated cells containing a lipid substance probably closely related to cholesterol. Comparing his material with that from two cases of Gaucher's disease he concluded that the pale vacuolated cells in the latter affection were also filled with a lipid substance and identical with the cells of his case. Brill and Mandlebaum, in 1913, were unable to demonstrate the presence of lipoids in the spleen in a case of Gaucher's disease, but their examination does not appear to have been complete. Lutz, in 1914, reported two cases of large-celled hyperplasia of the spleen pulp in diabetic lipemia, showing that his cases were similar to the one of Schultze; and that the large, pale, vacuolated cells were identical with those seen in the spleen in Gaucher's disease, but that the identity of the lipid-containing cells did not prove that the two processes were identical. Sapegno has also reported two cases of Gaucher's disease, concluding that the characteristic large pale cells show a deposit of lipoids, and are similar to the cells found in the spleen in diabetic lipemia. He also refers to a case of obstructive jaundice observed by Kramer in which masses of lipid cells were found in Glisson's capsule of the liver and in the splenic trabeculae.

Following these observations Anitschkow produced in rabbits by prolonged feeding of cholesterol the same picture of large pale vacuolated cells (cholesterol steatosis) in the spleen, lymph nodes and bone marrow as that seen in Gaucher's disease and diabetic lipemia. In 1916, Wahl and Richardson reported a study of Gaucher's disease in an infant eleven months old. The spleen, liver, and lymph nodes presented the usual picture of Gaucher's disease, but in addition the medulla of both adrenals was almost completely replaced by clusters of large pale vacuolated cells. Similar cells were found in the intestines, Peyer's patches, thymus, and adventitia of some of the smaller vessels. A thorough chemical and histological study was made of this case; and the writers con-

clude that Gaucher's disease is due to a disturbance of lipid and fat metabolism resulting in the accumulation of lipid substances in the cytoplasm of large pale cells that are chiefly reticulo-endothelial cells of the spleen, lymph-nodes, bone marrow, liver, Kupffer stellate cells, etc. These cells are concerned in the metabolism of the fats and lipoids and comprise the "endothelial-Stoffwechselapparat." In advanced cases specific parenchymal cells also take up the lipoids. There is not only a marked increase in the lipin content, but a marked alteration in the normal relations of the lipins. The fixed fats are greatly reduced, and the lipoids, such as lecithin and cholesterol, greatly increased. A lecithin-like body predominated in their case. Gaucher's disease therefore must be classed with the group of xanthelasmic conditions which are characterized by a more or less diffuse accumulation of lipoids in reticulo-endothelial or fibroblastic cells in one or more organs. It is a more diffuse and widespread involvement of the "endothelial-Stoffwechselapparat" than the cases of large-celled hyperplasia of the spleen seen in diabetic lipemia.

A clinical and pathological study of two cases of Gaucher's disease in infants reported by Knox, Wahl and Schmeisser, in 1916, includes the case given above and the sister of that case, aged fifteen months. The pathological findings in the second case were similar to those of the first; the essential feature being the occurrence, particularly in the hematopoietic system, of large pale vacuolated cells containing a peculiar refractive substance having the chemical and staining properties of lipid material. These writers regard their two cases as identical with the cases reported as Gaucher's disease, and conclude that this affection "is not primarily a disease of the spleen, or of any other organ, or set of organs, but is a generalized process due to a disturbance in fat metabolism, manifesting itself by lipid metamorphosis, that is, by the more or less diffuse accumulation of lipid material in many cells with the formation of the characteristic large pale cells. This process is most prominent in the hematopoietic system, especially in the spleen and lymph nodes, organs that are said to play a not unimportant role in abnormal fat metabolism."

If these conclusions as to the nature of Gaucher's disease are correct, an entirely new field of pathologic metabolism has been opened up, aside from the elucidation of the nature of the mysterious large pale cell characteristic of this process. As our knowledge of the lipid bodies is so incomplete any new light thrown upon fat and lipid metabolism becomes particularly valuable at this stage. Through the study of such pathologic disturbances of lipid metabolism we may gain important knowledge of the normal metabolism. Some progress into this field has already been made. The part played by the adrenals and spleen in lipid metabolism has already been pointed out by several writers. Krylow believes that the adrenal cortex regulates lipid metabolism, and that any insufficiency on its part would lead to an accumulation of lipoids in other parts of the body. Chalатов, Soper, Kusunoki, and others have pointed out the importance of the role of the spleen in lipid metabolism. Soper holds that the spleen's apparent large part in this is due to its extensive reticulo-endothelial system. The liver also plays a very important part in the regulation of cholesterol metabolism. By some writers it is placed between the adrenals and the spleen and endothelial apparatus in its relative importance in so far as this function is concerned. Other organs and

tissues (sex-glands, panniculus, etc.) may play an important role in the maintenance of cholesterol equilibrium, but we as yet have no definite knowledge of such metabolic processes. Increase of cholesterol in the blood has been noted in pregnancy. Injection of cholesterol emulsions in tumor-grafted rats has appeared to increase the rate of tumor-growth (Burnett and Robertson). Luden has found high cholesterol values in patients suffering from malignant disease, and on very insufficient grounds has advanced the hypothesis that cholesterol retention due to insufficient conversion or deficient elimination may be a primary factor in the etiology of malignant disease.

All forms of disturbances of fat metabolism should be studied in the attempt to add knowledge to this subject, particularly those of a generalized character. I wish to present here such a study in a field as yet untouched, the marked disturbances of fat-metabolism occurring in hypophysis disease (dyspituitarism). Two autopsy cases of this condition showing the two stages of this affection, one of adolescent dystrophia adiposo-genitalis, the other of dyspituitarism in an adult following an acromegalic stage, have occurred in my service. Both cases presented the striking obesity characterizing hypopituitarism. As far as I have been able to discover no studies have as yet been made of the character of the fat in this condition. Cushing, in his book on "The Pituitary and its Disorders" remarks (page 258) that "The adiposity of hypopituitarism is a generalized one—not limited solely to the panniculus—though its bedside recognition is necessarily restricted to the character of the subcutaneous disposal. The fat shows postmortem, certain peculiarities of color and consistency which suggest a different chemical composition from 'normal panniculus,' and is worthy of a differential analysis. It furthermore invades the organs, and in the liver particularly, there is often an extraordinary replacement of the cells by fat globules." In his description of the changes in the adrenals (page 281) he says: "As is true of the experimental states of hyperpituitarism [?], so in the clinical cases the adrenals which we have had an opportunity of studying histologically have shown an abnormal vacuolization (a lipoid change?) of the cells of the zona fasciculata of the cortex (Fig. 313)." In the legend of this figure the condition is correctly stated as hypopituitarism, evidently a typographical error in the text, and the adrenal pictured shows a condition of marked liposis.

#### CASE I.

H. F., Schoolboy, aged 18 years. American. Admitted to Neurologic Clinic (Dr. Camp), 1-16-12.

CHIEF COMPLAINT.—Failing vision.

FAMILY HISTORY.—Negative. Two brothers and two sisters, living and well.

PREVIOUS PERSONAL HISTORY.—Has had measles and smallpox. No history of injury. No bad habits. Has had some headaches ever since he can remember, accompanied by nausea and occasional vomiting.

PRESENT TROUBLE.—About one year ago the headaches became more severe and constant. He then had convulsions, and was unconscious for two weeks (with spasms during first week). No fever. Eyes began to fail two years ago. Since the attack of unconsciousness his headaches have been less severe. At present he complains chiefly of a difficulty in vision and constant sleepiness. Is in the 11th grade at school. Seems as bright mentally as the other pupils.

**PHYSICAL EXAMINATION.**—Very obese boy. Height 4 ft., 10½ in. Weight, 120¾ lbs. Panniculus hangs in long folds from breast and abdomen. Feminine type. Well developed mammae. Face rounded and full. Skin of good color. No axillary hair; very little pubic hair. Neck very short with adipose collar. Thyroid not enlarged. Genitalia very poorly developed, hardly visible. Right testicle undescended. Hands and feet short and stubby. No enlarged glands. Heart sounds very faint and irregular. Mentality good. Facial expression drowsy. Occasionally a choreiform movement in the extremities. Can count fingers at about five feet. Reflexes diminished. Prickly sensations at times over the skin, particularly in thighs. Urine negative. R.b.c., 4,370,000; w.b.c., 12,050; hemoglobin 90. X-ray examination shows a dense mass about the size of a hazel-nut in the anterior portion of the sella. Ophthalmologic examination showed well-advanced optic atrophy. Blood-pressure 105.

Operated by Dr. Canfield 2-9-12. Took anesthetic badly. Route through the sphenoidal cells removing part of the floor just anterior to the anterior wall of the sella. Through the opening meninges and brain bulged. This was punctured and about two drams of blood and fluid escaped. Operation closed without removal of hypophyseal tumor. Patient recovered consciousness, and remained so for several hours without pain; then became delirious, with spasms of eyes and face, great restlessness. Temperature per rectum 104°. Became gradually semi-conscious. No paralysis. No pain. Developed spasms before death at 6 A. M. next morning. The autopsy was made five hours after death.

**AUTOPSY PROTOCOL.**—**EXTERNAL EXAMINATION.**—Body of a very obese boy, apparently about ten to twelve years old. Length 150 cm. Somewhat feminine in type. Breasts very fat; no fluid can be expressed from them. Hands and feet short and thick. Abdomen above level of thorax, large folds of panniculus. Face has a rather stupid adenoid expression. Very fat and rounded. Marked fatty collar. Hair of head is abundant, but there are few axillary and pubic hairs; these are fine and light in color. Both testicles are in the small scrotum; they are about the size of those of a ten year old boy. Right one is smaller. Short retracted foreskin; glans normal. Mucoud secretion dripping from meatus. Panniculus throughout very thick and firm. No edema except slight over ankles. Muscles poorly developed. Rigor mortis only in muscles of jaw. Body is very warm, feels hot to the touch. Teeth negative. Nostrils are dilated, and contain clots of blood. Arms and legs show a number of bruises. Hypostasis is marked, purplish red. Sacrococcygeal dimple is very marked. Percussion of thymus region shows an increased area of dullness.

*Spinal Cord.*—Not removed.

*Brain.*—Scalp and periosteum negative. Skull-cap is thin, particularly over the vertex where there are well defined areas of deficient ossification, and also in the region of the fontanels. Dura is firmly adherent all over, and increased in thickness along the longitudinal sinus. Intracerebral pressure greatly increased. Meningeal and pial vessels are markedly congested. Meninges over frontal lobes are covered with fresh blood-clot; and there is an extravasation of blood through the subarachnoid space. On lifting the frontal lobes there is seen a large recent clot between the base and the optic nerves. Blood clots in left temporal and occipital regions. Brain is adherent to the cranial floor in the neighborhood of the

hypophysis. On separating the attachments the softened brain substance in the neighborhood is much torn. It is discolored and partly necrotic and hemorrhagic. A large clot extends between the frontal lobes along the left anterior cerebral. The softening of the brain substance and hemorrhage are most marked in the neighborhood of the chiasm where the basal dura is torn and the floor of the skull penetrated by the operation anterior to the hypophysis. No trace of this organ is visible. The sella is not greatly enlarged and is filled with a projecting mulberry mass, very hard and giving a clear ringing sound when struck. In the floor of the cranium just anterior to the sella is a similar hard nodule the size of a peppercorn. From the hard mass in the sella an atypical softer tissue infiltrates the discolored and softened brain tissue lying above it. The basal arachnoid and pia are much thickened and covered with recent clots. Ventricles dilated, contain a blood-stained fluid. Pineal gland normal in size and appearance. Choroid plexus negative. Cerebral and cerebellar tissues soft, edematous and congested.

*Main Section.*—Abdominal panniculus 5 cm. thick; that over thorax is 3 cm. It differs in appearance from normal fat; when first cut into it is very glistening, translucent, very firm and is light buffy-yellow in color. The panniculus is very warm to the touch, steaming. On cooling it becomes more buff in color, opaque and very hard. The lobules are much larger than normal panniculus. The appearances suggest a myxedematous fat. The subserous panniculus is 3 cm. thick and of the same character. Omentum and epiploic appendages, mesentery and subserosa of bladder show similar thick deposit of fat. The abdominal and thoracic muscles are small and show abundant fatty infiltrations. No free fluid or gas in peritoneal cavity. Liver very large extending 3 f.b. below ensiform, and 2 f.b. below edge of ribs in right nipple line. Spleen not enlarged. Diaphragm 5th rib on left; 4th on right.

*Thorax.*—No fluid or gas in thoracic cavity. Lungs free. Apex of heart in 4th intercostal space, left parasternal line. Mediastinal fat very abundant. Thymic region filled with a large pink, two-lobed thymus, the left one extending over left ventricle to apex, the right one covering the right auricle. The upper boundary of the lobes touches the thyroid. Pericardium normal. Heart normal size; undersized if amount of panniculus is taken into account. This is very abundant. Right ventricle contains large red and white clot. Left ventricular wall is 10 mm. thick. Mitral flaps and orifice negative. Pulmonary artery and aorta negative. Foramen ovale and ductus arteriosus closed. The lungs show marked congestion and numerous small hemorrhages. The intima of the ascending portion of the aorta shows patches of fatty degeneration.

*Neck Organs.*—Thyroid is small. On section shows but little colloid, resembles foetal thyroid. Homogeneous surface. Four brownish-pink parathyroids. Not enlarged. Cervical glands not enlarged.

*Abdomen.*—Spleen weighs 50 gms. Soft. Capsule negative. Follicles numerous, large. Cut surface shagreened. Large intestine distended; unusually large; otherwise negative. Stomach negative. Pancreas normal in size and consistency. Much fat about it. Both adrenals are large, absolutely and in proportion to size of kidneys. Cortex yellow, medulla hypoplastic, much fat about both adrenals. Kidneys have enormous fatty capsules. Fibrous capsules strip easily. Fetal lobulations well preserved. On section kidneys show



marked congestion. Pelvic fat much increased. Liver weight 1030 gm., capsule negative. Surface mottled, yellowish and red, with fatty shine. Lobules not distinct. Mesentery very fat. Mesenteric and retroperitoneal nodes not enlarged.

*Genitalia.*—The testes are surrounded by a fatty sheath. On section they appear as undeveloped infantile testes. Prostate cannot be felt or seen. Seminal vesicles not developed. Other organs negative.

*MICROSCOPICAL EXAMINATION.—Tumor of Hypophysis Region.*—Presents the appearance of an adamantino-carcinoma with hyaline and calcareous masses of a dentine-like substance arranged in mulberry masses or agglomerations of rounded concretions. These are formed by the hyaline transformation of cords and strands of large squamous prickles-cells. Cords of these cells infiltrate the brain substance after the manner of a carcinoma; those in the brain for the greater part showed no calcification, and the cell-cords consists chiefly of a central hyaline mass around which cells with very large intercellular bridges or prickles are grouped showing the characteristic palisade arrangement at the periphery of the hyaline mass. Small cords show no hyaline change. The brain tissue about the tumor-cords shows atrophy with marked gliosis, and occasional cellular infiltration. A small area of hypophysis tissue corresponding in structure to the pons anterior was found showing the same adamantinoma infiltration.

*Thyroid.*—Many follicles contain a thin colloid; but the majority contain none. The organ closely resembles the fetal thyroid.

*Parathyroids.*—These were hypoplastic and showed fatty infiltration.

*Thymus.*—Hyperplasia of the medulla; atrophy of cortex.

*Heart.*—Fatty infiltration.

*Aorta.*—Diffuse fatty degeneration of the intima, with small raised patches of lipid cells resembling those of Gaucher's disease.

*Lungs.*—Marked congestion and edema; hemorrhages; acute purulent bronchitis and bronchopneumonia.

*Spleen.*—Marked congestion. Large follicles with lymphoid exhaustion. Sclerotic arterioles. Scattered lipid cells, not numerous.

*Stomach.*—Negative.

*Intestines.*—Excessive mucus formation.

*Pancreas.*—No changes noted.

*Mesenteric and Retroperitoneal Nodes.*—Lymphoid exhaustion.

*Adrenals.*—Abundant chromaffinic cells. Hypoplastic medulla; cortex hypertrophic with marked but irregular liposis, involving in some regions the glomerular zone, in others the fascicular. Marked hypertrophy of some of the fascicular cords.

*Kidneys.*—Cloudy swelling and congestion. Numerous glomerular scars. Scattered lipid cells in lymphatics about large veins.

*Testes.*—Infantile in type, with vacuolation of germinal cells. Interstitial cells hypoplastic.

*Liver and Panniculus.*—Will be described below with that of Case II.

*PATHOLOGICAL DIAGNOSIS.*—Infantilism, dyspituitarism adiposo-genitalis; adamantino-carcinoma of hypophysis; hyperplastic thymus; lymphatic constitution; general lipomatosis (liposis) most marked in liver (cholesterol steatosis); intraperipheral zonal necrosis of liver; hypoplasia of thyroid and adrenals; post-

operative hemorrhage and operative trauma at base of brain; acute purulent bronchopneumonia (aspiration).

#### CASE II.

J. B. M., age 29 years; sailor by occupation. By birth American. Admitted to Neurologic Clinic (Dr. Camp), March 9, 1912.

CHIEF COMPLAINT.—Disturbances of vision, staggering, mental disturbances.

FAMILY HISTORY.—Negative. No case of tuberculosis, cancer, or nervous disease.

PERSONAL HISTORY.—Always well until present trouble began. Mentality always good. Has lived an out-of-doors life. Three years ago began to have violent headaches every few days, lasting 1-2 days and followed by vomiting. After two years these stopped suddenly. At this time he began to complain "of not feeling well," but had no well-defined symptoms. Slept a great deal, all night and during the day except when wakened for meals. Would fall asleep on duty. Suddenly noticed a peculiar tendency to lean to the left when walking, as well as a peculiar manner of carrying left arm. Developed hoarseness and a hesitating slow speech, with difficulty in making himself understood. Lost interest in everything, and avoided particularly reaching for things, asking others to do this for him. Lost his skill in marksmanship and his interest in sports. The difficulty in walking has steadily increased, as have choking spells induced by eating. His sister has noticed nothing strange about his mentality except a slight childishness. During the last year he has had five attacks of dyspnea, hard chills, hands drawn up, side of face drawn up, and fever running as high as 103.5°, followed by vomiting. Cannot walk well after attacks, and has fallen several times. At the present time he has to be dressed and fed. Cannot walk out alone. Smokes excessively; denies alcoholism and venereal disease. No note made of sexual state. Last May was examined by Dr. Parker in Detroit who found  $\frac{1}{2}$  vision in each eye. Was x-rayed at this time.

PHYSICAL EXAMINATION.—(March 11, 1912, by Dr. Camp). Large build, well nourished, wears a No. 8 shoe. Hands are large, but patient says they have always been so. Head is fairly large; normal shape. No acromegalic jaw. Thyroid not enlarged. Mentality seems fairly clear. Tendency to senseless jokes. Slight clouding of consciousness; memory is defective except for more recent events. No delusions; no mental retardation. Replies are prompt, although speech is slow. Left pupil reacts to light; right does not. Neither pupil reacts to accommodation. Some defect in lateral motion, particularly to the right. Eye-balls do not converge. Temporal hemianopsia in left eye; in the right eye some contraction of the visual fields in all directions, but no distinct hemianopsia. Palpebral fissure a little wider on left side than on right. Right side of face drawn up distinctly better than left. Tongue straight, no tremor, no atrophy. No ataxia; no intention tremor; no hemiasynergia in hands. Slight staggering gait with eyes closed. Sensory tests normal. Reflexes prompt and equal. Examination of heart, lungs and abdominal organs negative. Urine negative. Lumbar juncture negative. Wassermann negative. Patient was examined again on June 29, 1912, by Dr. Camp. Condition about the same. Returned December 2, 1912. Examination by Dr. Camp. Had severe headaches after leaving the hospital in June;

one until yesterday when he had a severe attack with dizziness. Fell heavily, but was not unconscious. Says that he was paralyzed on the left side four months previously. Symptoms about the same as before. Blood and spinal fluid gave negative Wassermann. Headaches increased. Patient feels "all in." Examination by Dr. Canfield revealed the presence of a nasal tumor. Operated on December 1 by Dr. Canfield. Submucous resection of the septum showed a tumor mass extending from the sphenoid cavities from regions back of the sphenoid down into the septum. The tumor was very soft, dark grayish in color, and flecked with white spots. Microscopical examination of this in our laboratory showed the neoplasm to be a large round cell sarcoma containing numerous calcareous concretions (psammoma). Regarded as probably primary in basal meninges.

After the operation condition continued unchanged (headache, sleepiness, etc.). Temperature rose to  $105^{\circ}$  the day after the operation. On January 16th he complained of more or less constant pain through temples. Seen at intervals of several weeks. Irrational at times. By April 9, 1913, enlargement of hands was notable. His sister said that this had occurred during the last few weeks. Sleepiness increased. By May 19 his face and head looked distinctly larger; appearance of hands typically acromegalic. Feet not grossly enlarged. Chief complaint still headache and "sour stomach." Anxious to have another operation because of "untold agony" in head. Glucose (100 gms.) test negative. Acromegalic condition increased, slowly until January, 1914, when his sister noticed that his shoes were looser. Complained at this time of chilliness; skin of hands mottled and discolored. Tonic convulsive movements in upper extremities. Temperature rises to about  $103^{\circ}$  after chill. Subcutaneous panniculus all over the body increased. Secondary type of optic atrophy in right eye. Fundus of left is more congested than right. Headaches have ceased. No note of sexual state occurs in the history. Patient showed gradual cessation and subsidence of the acromegalic condition with increasing symptoms of hypopituitarism (increase of panniculus, etc.) until his death on April 15, 1914, at 7:50 A. M. Autopsy at 10 A. M.

**AUTOPSY PROTOCOL.**—Body large; masculine type; length 175 cm. Acromegalic facies and hands. Obese; panniculus abundant all over body. Body and head hair abundant. Genitalia well-developed; testicles soft and flabby. Body very warm; axillary temperature  $99^{\circ}$ , rectal  $104^{\circ}$ . No rigor mortis. Moderate purplish hypostasis.

*Spinal Cord.*—Not examined.

*Head.*—Scalp and periosteum negative. Skull-cap thickened; inner surface rough, gray, meningeal grooves prominent. No exostoses. Pacchionian depressions shallow. Dura sclerosed. Dura not thickened but adherent throughout. No osteomata in dura.

On lifting and removing the brain a large soft tumor is found at the base, in the interpeduncular space, extending more into the base of the right cerebral hemisphere than on the left. Smaller lobulated masses are found about the periphery of the larger mass. The tumor has destroyed the right optic tract and displaces the 5th and succeeding cranial nerves backward. The third nerve appeared to pass through the tumor. The pons was compressed by the tumor to half its normal thickness. On the right the growth extended into the right temporosphenoid

noidal lobe by two finger-like projections, one in the region of the right lenticular body, the other into the third ventricle. The internal capsule was compressed between them. Tumor was reddish, soft, well encapsulated toward the brain substance, but ragged and torn below where it had been separated from the tumor mass extending through the floor of the skull into the retropharynx. Anterior to the sella there was an opening about the size of a silver dollar through which a tumor-mass about the size of a tennis ball extended into the nasopharynx. Base of skull about the opening was soft, necrotic and infiltrated with extensions of the growth. The anterior portion of the sella was destroyed, and no trace of hypophysis tissue could be made out, the entire organ apparently having been destroyed by the growth.

*Main Section.*—Abdominal panniculus is 5 cm. thick, thoracic 3 cm. The fat is in large coarse lobules, unlike normal fat, pale lemon-buff in color, firm, translucent and shining; but becoming darker and opaque on cooling. The panniculus is very warm to the touch. Omentum is rich in fat of the same type; likewise the epiploic appendages and mesentery. Abdominal organs very hot, almost bloodless. Intra-abdominal temperature 107.1°. Tissues dry very quickly on exposure to air. No free fluid in cavity. Lower border of liver at ensiform in mid-line, a hand's breadth above the lower edge in right nipple line. Slight rigor mortis in abdominal muscles. Diaphragm at 5th ribs.

*Thorax.*—No fluid in thorax. Apex in left parasternal line behind 5th rib. Lungs meet anteriorly above. No pleural adhesions. Fat tissue in anterior mediastinum very abundant, of same appearance as the panniculus. Large lobules of thymic fat in which remains of thymus are present. On section thymic fat is diffusely pink. Heart is negative, save for a dilatation of the right side, an adherent thrombus in the right auricular appendage, increased subpericardial fat, brown atrophy of muscle and a thickening of the endocardium of the left auricle. Aorta showed early sclerosis in the ascending part of the arch. Lungs show passive congestion. Entire lower lobe of right lung is airless, solid, early stage of red hepatization.

*Mouth and Neck.*—Hyperkeratosis of tongue (brown hairy tongue). Papillae at root hyperplastic. Larynx, trachea and esophagus negative. Thyroid is slightly larger than normal, colloid is not diminished. Parathyroids hyperplastic, 8 mm. in diameter, purplish in color. Cervical hemolymph nodes hyperplastic.

*Abdomen.*—Spleen weighs 120 gms. Small, soft, shows few follicles on section.

*Stomach and Intestines.*—Dilated, otherwise negative.

*Pancreas.*—Fatty infiltration of tail. Soft.

*Liver.*—Very hot to the touch. Clinical thermometer inserted showed five hours after death a temperature of 108°. Small in size, weighs 1730 gms.; sharp edges; mottled surface; yellowish red; opaque fatty shine.

*Mesentery* is very rich in fat, arrayed in coarse lobules, lemon-yellow color, appearance same as that of panniculi. Fat changes color on exposure to air, becoming a diffuse pink in many areas, giving it a peculiar mottled appearance.

*Adrenals* are very large, 8 cm. long, 5 cm. broad, and 4 mm. thick. Surrounded by dense masses of fat and old fibrous adhesions.

*Kidneys* have very large fatty capsules. Fibrous capsule strips easily on the

left, somewhat adherent on the right. On section kidneys show slight atrophy and congestion. Retroperitoneal lymph nodes atrophic, show fatty infiltration. Thoracic and abdominal muscles show fatty infiltrations.

*Pelvis.*—Penis negative. Left testis rather pale. No increase of stroma visible to the naked eye. Right testis pale, soft. Prostate smaller than normal, congested. Seminal vesicles very much dilated with a thin watery fluid. Walls negative. Bladder and rectum negative.

*MICROSCOPICAL EXAMINATION.*—Sections of neoplasm from base of brain and nasopharynx show it to be a round cell angiosarcoma with numerous psammoma concretions scattered through it. It bears no resemblance to the hypophyseal struma. In the sella turcica region there were found areas in a number of sections of a typical hypophysis adenoma or struma (chromophobe type) infiltrated, compressed and destroyed by the sarcoma. The appearances lead me to believe that a typical acromegalic hypophyseal struma has been either the seat of a malignant change, or what is more likely has been destroyed for the greater part by a sarcoma arising primarily in the basal meninges or in the floor of the skull. The penetration of the latter, the early growth downward into the nasopharynx and septum support the latter view. The neighboring brain structures show pressure atrophy and necrosis, gliosis and sarcoma infiltrations.

*Tongue.*—Hyperkeratosis.

*Thyroid.*—Colloid hyperplasia.

*Parathyroids.*—Hyperplasia.

*Cervical Lymph Nodes.*—Marked lymphoid hyperplasia and congestion. Large germ centers with exhaustion of the lymphocytes. Sinuses crowded with macrophages, many of these are hemophages containing red cells or pigment, many of them are filled with fine fat droplets (lipoid cells). Apparent very active hemolysis.

*Lungs.*—Acute hypostatic pneumonia. Edema and chronic passive congestion.

*Heart.*—Brown atrophy, fibrosis, fatty infiltration and degeneration; slight cloudy swelling.

*Thymus.*—Remains of thymus in fat tissue showing newly-formed cords of lymphoid tissue along lymphatics distended with macrophages and hemophages. Hassall's corpuscles small and few.

*Spleen.*—Chronic passive congestion, atrophy. Few scattered lipoid cells. Numerous hemophages.

*Stomach and Intestines.*—Acute catarrh. Appendix contains fecal concretion. Old appendicitis.

*Pancreas.*—Fatty infiltration. Very large fat cells with greenish coloration, showing mixture of lipoids. Hypertrophic islands of Langerhans. Hyaline substance in many acini.

*Liver.*—See below.

*Adrenals.*—Marked hypoplasia of medulla. Small amount of chromaffinic tissue. Practically the entire cortex shows a marked liposis of the parenchymatous cells. The liposis is most marked in the glomerular and fascicular zones. Hypertrophic fascicular cords are also numerous. (See further below.)

*Kidneys.*—Cloudy swelling and fatty degeneration of the tubular epithelium.

Areas of chronic and subacute nephritis. Numerous casts. Lipoidosis of tubular cells.

*Aorta*.—Early sclerosis. Fatty degeneration of intima. Small elevations made up of collections of lipid cells in intima.

*Mesenteric and Retroperitoneal Lymph Nodes*.—Hyperplasia, lymphoid exhaustion, sinus catarrh, great numbers of macrophages and hemophages. New formation of lymphoid tissue throughout mesenteric and retroperitoneal fat. Many lipid cells.

*Hemolymph Nodes*.—Hyperplasia. Sinuses crowded with hemophages containing blood cells and pigment. Scattered lipid cells.

*Testes*.—Aspermatogenesis. Stroma increased. Basement membranes thickened. Fatty degeneration (liposis) of germinal epithelium.

*Prostate*.—Moderate glandular hyperplasia.

*Seminal Vesicles*.—Negative.

**PATHOLOGICAL DIAGNOSIS**.—Hypopituitarism following acromegaly (sarcoma replacing hypophysis struma); sarcomatous infiltration of brain, base of skull, nasal and pharyngeal cavities; general obesity (general liposis); cholesterol steatosis of liver and adrenals; intraperipheral zonal liver-necroses; postmortem hyperpyrexia; hyperplasia of adrenal cortex, hypoplasia of medulla; hyperplasia of lymphatic and hemolymph nodes with hemolysis; hypertrophy of islands of Langerhans; persistent thymus; lymphoid exhaustion of germ-centers; subacute parenchymatous degenerative nephritis; acute hypostatic pneumonia; acute gastrointestinal catarrh; hyperkeratosis of tongue.

#### ANALYSIS OF CASES.

These two cases of hypophysis disease present several striking features in common. They are both examples of hypopituitarism. Case I, with deficient hypophysis secretion from the beginning; Case II, with an earlier stage of hyperpituitarism (acromegaly) succeeded by a period of hypopituitarism following the destruction of the primary hypophysis adenoma (struma). Although, as Cushing has strongly emphasized, cases of hyperpituitarism sooner or later pass into a second stage of hypopituitarism, it seems reasonable to suppose that the transformation in this case is explainable upon the apparent grounds found. Leaving the hypophyseal features of both cases and the acromegalic symptoms of Case II out of the question, the general picture in both cases is the same: a marked obesity or liposis involving all organs, particularly the panniculi, liver and adrenals, with scattered lipid cells elsewhere. The atypical character of this fat was shown best in the liver and adrenals, and it was most thoroughly studied in the former organ.

**LIVER CHANGES IN CASES I AND II**.—Both livers show identical changes, perhaps more marked in Case I, although portions of the liver in Case II show as marked changes as any in the liver of Case I. These changes will now be described in detail.

*Hematoxylin and Eosin Preparations*.—Sections from blocks imbedded in paraffin and stained with hematoxylin and eosin give on first glance the picture of a fatty liver in which the processes of fatty infiltration and fatty degeneration are combined. The lobules are smaller than normal. The cells of the central liver-

zone show numerous fine droplets or vacuulations, giving the cell the characteristic "ground-glass" appearance. Through the intermediate and peripheral zones large fat droplets appear, not regularly as in an ordinary fatty liver, but arranged in more or less well defined groups. They are, however, most numerous toward the outer portion of the intermediate zone, and not at the periphery of the lobule. Groups of such large vacuoles occur also in the central zone. Small fat droplets are found also in all of the liver cells except in certain peculiar zones or bands that run around the lobule in the inner portion of the peripheral zone. In these areas there are few fat droplets, the liver cells are small, granular, necrotic or replaced by a cellular fibroblastic area in which remains of liver cells can be made out. In a few lobules these fibroblastic zones touch the periportal islands, but

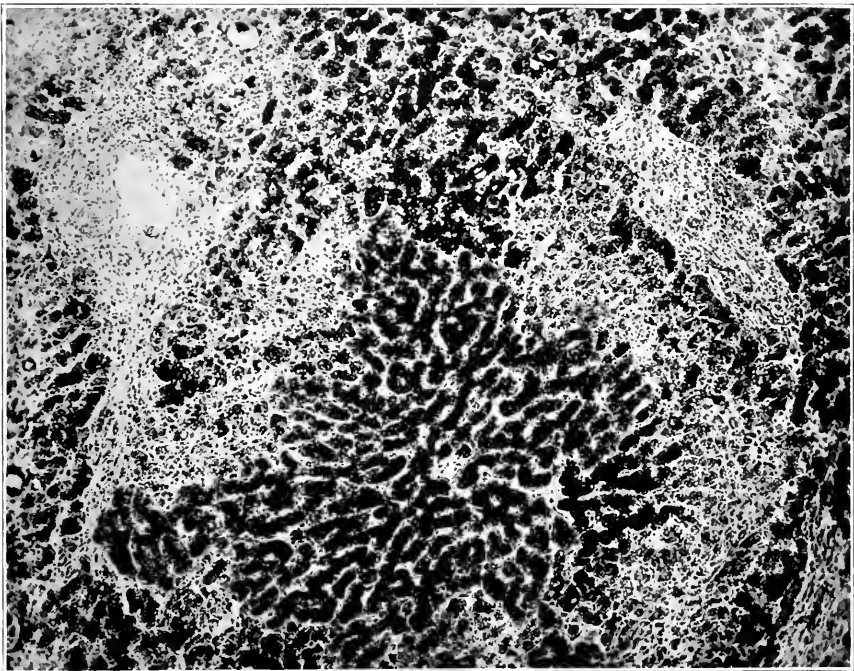


Fig. 1.—Cross-section of liver lobule from liver of Case I. Frozen section, Sudan III and hemalum stain. Central zone and portion of mid-zone and narrow border of peripheral zone show liver-cells filled with minute anisotropic droplets staining deep brownish red, with larger scattered isotropic droplets staining deep yellow-red. Inner portion of peripheral and outer portion of mid-zone show peculiar necrosis with fibroblastic proliferation (intraportal necrosis and beginning cirrhosis).

usually a narrow zone of liver cells filled with fine fat granules separates this zone from the trabeculae. This picture seems to me to be quite unique. I have never seen anything like it in any other liver but in these two cases of hypopituitarism. The periportal tissue is cellular and fibroblastic, frequently showing newly formed bile ducts in numbers. The appearances are those of an early stage of a peculiar form of cirrhosis. It shows more distinctly in the H. and E. preparations of Case I than of Case II; but the peculiarity of this change is best revealed in the sections stained with Sudan III and Scharlach R. and counterstained liver with hemalum. The protoplasm of the outermost cords of cells stains most heavily and has the smallest number of fat droplets. The veins are dilated, but the intralobular

capillaries only moderately so. There is no picture of nutmeg liver or central necrosis, the liver cells around the central veins staining as well as those in the periphery as far as the nuclei are concerned. There is no necrosis, and no pigmentation of the liver cells of the central zone. Iron tests were negative. No pigmented stellate cells were found. Bile-ducts showed no changes.

#### MICROCHEMICAL STUDY.

*Osmic Acid.*—In frozen sections treated with one per cent osmic acid, or in sections so treated after formol-fixation the fat content of the liver cells was more decidedly revealed. The small droplets in the central zone of the lobule stained a lighter gray than the large fat droplets scattered irregularly. Some of the droplets are not acted upon at all by the osmic acid or show only the slightest gray tint

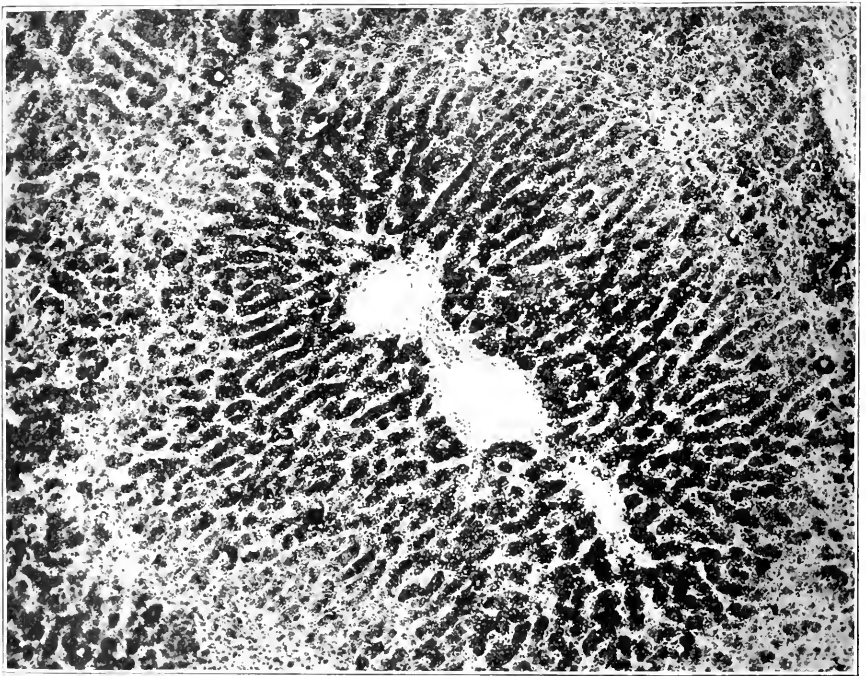


Fig. 2. Cross section of liver-lobule from liver of Case II. Frozen section, stained in Sudan III and hemalum. Central zone, greater portion of mid-zone and narrow outer portion of peripheral zone show liver-cells filled with fine anisotropic droplets staining deep brownish red, with scattered larger isotropic droplets staining deep yellow-red. Inner portion of peripheral zone and outer portion of mid-zone show the peculiar necrosis and early cirrhosis (intra-peripheral necrosis).

(cholesterol). The peculiar distribution of the fat in the two livers stands out very prominently in the osmic acid preparations. There appear in addition droplets of fat apparently in the lumina of the liver capillaries, and these were mistaken at first for free fat emboli in Case I. They are undoubtedly stellate endothelial cells showing a marked lipoidosis. These give a more gray color-reaction with the osmic. Osmic preparations of adrenals and aorta showed similar color reactions, a large part of the small droplets staining light gray. Sections of the panniculus stained deep brown black.

*Sudan III.*—Beautiful preparations were obtained with frozen sections



stained in an acetone solution of Sudan III and counter-stained with hemalum. The fine fat droplets in the central zone and throughout the liver-lobule stained a peculiar brownish-red, while the majority of the larger droplets stained an orange-red to brick red as does neutral fat (glycerin-esters). Other large droplets were light yellow or pale buff in color. Counterstaining with hemalum brought out the unique distribution of the fat in the liver-lobules. The fat-free areas or zones stand out in these preparations so strikingly and the cellular infiltration and proliferation is so prominently brought out that it is difficult to believe that one is looking at the same tissues seen in the paraffin-hematoxylin-eosin preparations. Occasional fat-droplets are found in these necrotic zones, and such appear to be chiefly in the stellate cells, although a few liver-cells con-

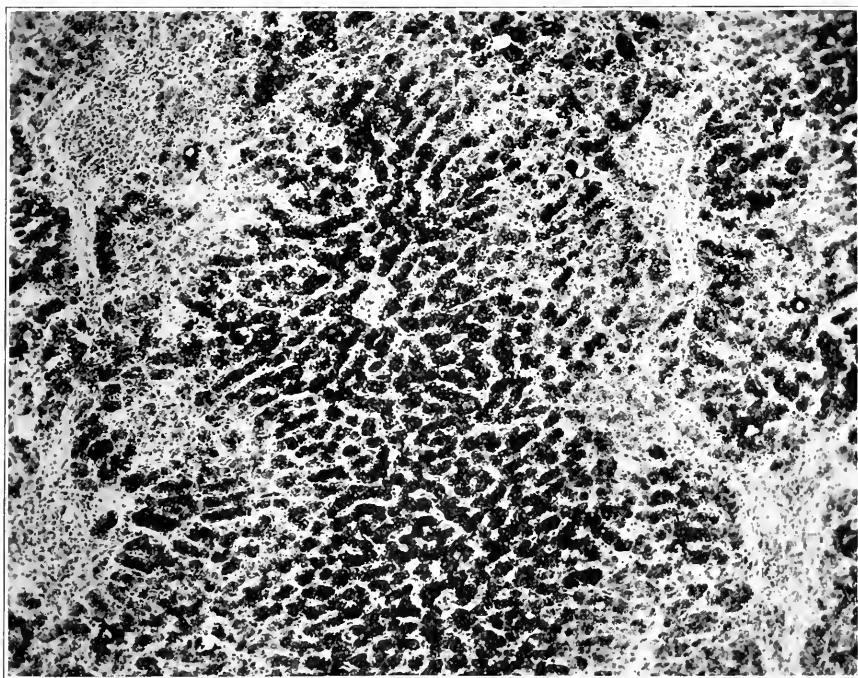


Fig. 3.—Tangential section of liver-lobules from liver of Case I. Frozen section, Scharlach R and hemalum stain. The small black granules in the liver-cells are brownish-red anisotropic fat-droplets (cholesterol-esters), the larger black droplets irregularly scattered are deep red isotropic fat-globules (glycerol-esters). The intraperipheral zone of necrosis and beginning proliferation is well shown.

taining large droplets occur also. The borders of these blue zones, as they appear in the hemalum preparations show a transition from liver-cells containing brownish red granules to cells containing fine brown granules. These brown granules are finer than the red ones and are scattered in the same manner through the cell. They do not give a hemosiderin reaction. Some liver-cells with red granules in one end and brown ones in the other are seen in the borders of these relatively fat-free zones. It is evident, however, that the lack of fat in these narrow zones is due chiefly to a necrosis or disappearance of the liver-cells in these areas, and to a replacement of these by a fibroblastic proliferation.

*Scharlach R.*—Similar results are obtained in frozen sections stained with an acetone solution of Scharlach R and counterstained with hemalum. The fine granules stain a deep red that has a distinct brownish cast, but their staining is not so intense as that of the large droplets which stain deep bright scarlet red without the brownish cast. The differential staining is about equal in the case of Sudan III and Scharlach R. I have noticed that students vary greatly in their ability to distinguish these differences with these two stains, some seeing it much better in the case of one, others with the other stain. Such results are probably ascribed to differences in color-perception due to individual training or experience in colors. With the Sudan III staining of frozen sections there seems to be at least ten times as much fat in the sections as in the case of osmic acid

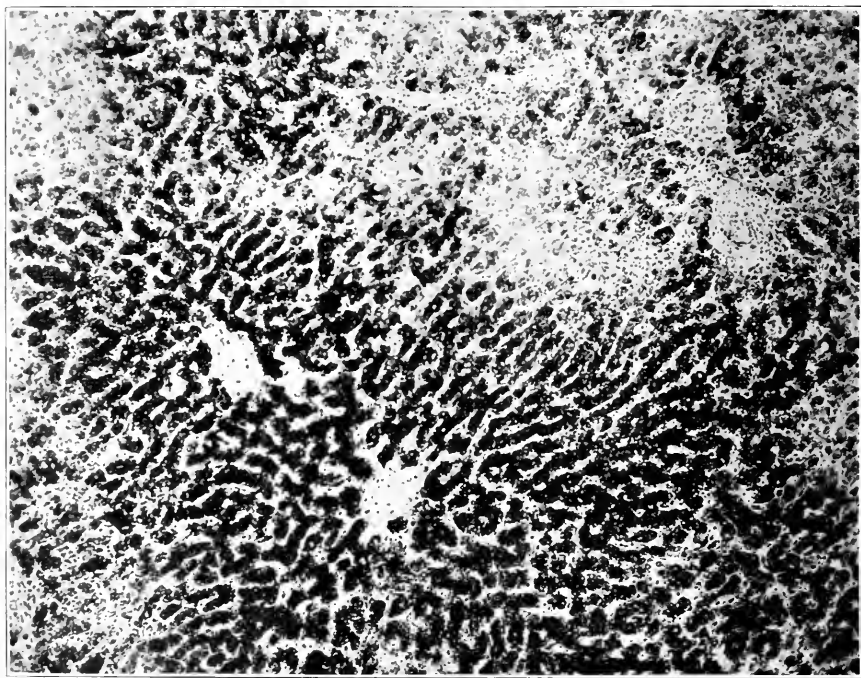


Fig. 4.—Tangential section of liver-lobules from liver of Case II. Frozen section, Scharlach R and hemalum stain. Same appearances as in Fig. 3.

staining. With Scharlach R the amount of fat seems still greater, but this is probably due to the greater intensity of color in the Scharlach R stain.

*Nile-blue Sulphate.*—With Nile-blue sulphate the fresh frozen sections showed a great variety of color reactions with the fat droplets. The majority of the larger droplets stained red, some pink, some pink-violet, some purple, others deep blue. The majority of the fine droplets stained pink or lavender. The fine droplets in the adrenal cortex and in the lipoid cells elsewhere stain pink or lavender to violet or blue.

*Weigert's Hematoxylin.*—Deep blue to blue-gray droplets are shown in the larger fat cells. When counterstained with van Gieson's the blue droplets appear as darker brown droplets in the ochre-colored protoplasm.

*Indophenol.*—In sections stained with a saturated solution of indophenol

in 70 per cent alcohol and contrasted with lithiumcarmin good fat preparations were obtained without any differential staining.

*Benda's Method.*—This gave no differential staining. Stains for fatty acids and soaps negative. Other staining methods (Smith-Dietrich's, etc.) were negative as far as differential staining was concerned.

*Optical Appearances.*—With polarized light doubly refractile (anisotropic) droplets were numerous in all the cells containing fine fat droplets. The larger cell droplets or crystals were chiefly isotropic, but anisotropic droplets were present in all cells containing fat. Crystal-like bodies appeared in the droplets on freezing. Warming caused the doubly refractile droplets to disappear.

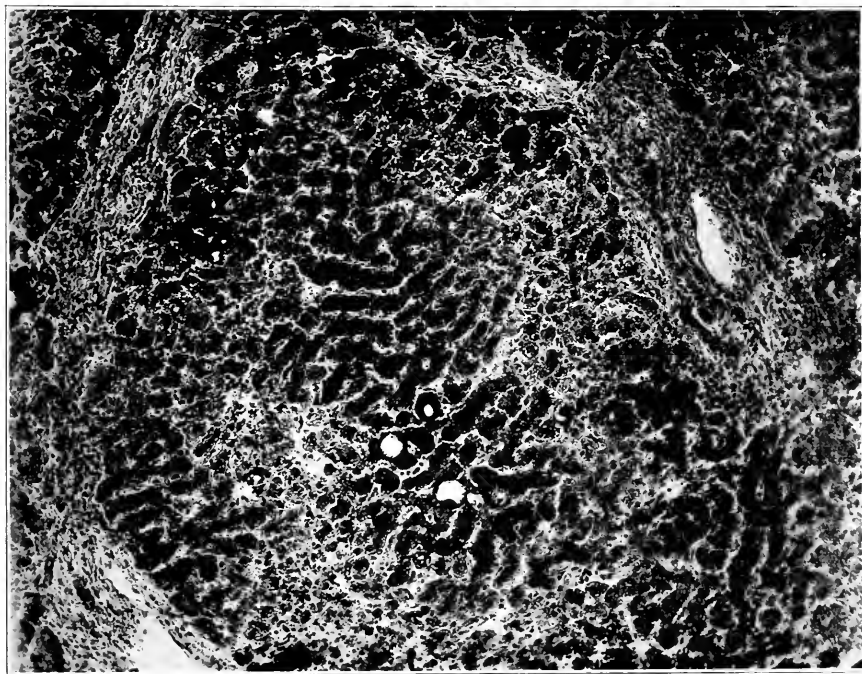


Fig. 5.—Section of liver lobule from liver of Case 1. Frozen section, Scharlach R and hemalum. Slightly higher magnification. Shows same character of lipin changes as in preceding figures, but in addition a more marked degree of peripheral necrosis and proliferation (more advanced cirrhosis).

Alcohol, ether, xylol and hot paraffin dissolved all of the droplets from the cells leaving vacuoles. Acids and alkalis did not dissolve them. Chromate fixation caused the larger cell droplets to become brown or greenish. No effect was noted upon the finer ones. Formol fixation had a marked effect upon the largest fat droplets in the panniculi, causing a formation of yellowish or green needle-shaped crystals in the cell. Portions of the two livers have been kept in formol and given each year to laboratory classes for fat study. While the differential reactions shown by Scharlach R, Sudan III and osmic acid are still obtainable they are not as sharp as at first; and the Nile-blue sulphate reactions have become very capricious. At present many droplets stain blue or purplish blue. Long-continued residence in formol undoubtedly affects the fats of the

cells. The most accurate conclusions therefore would be those drawn from the study of perfectly fresh tissues.

From the fat-reactions shown above it is evident that the livers of these two cases of hypopituitarism present an unusual lipin content. The presence of numerous anisotropic droplets, staining a lighter gray with osmic acid, brownish or yellowish red with Sudan III and Scharlach R, and pale pink with Nile-blue sulphate make it very probable that these are cholesterol-esters. Other lipoids may be mixed with these and with the glycerin-esters in these droplets. The lipin combination may be very complex. It is quite evident that our microchemical tests at the present time are not sufficiently reliable to differentiate accurately the different lipoids. We were unable to make satisfactory chemical studies of these lipins

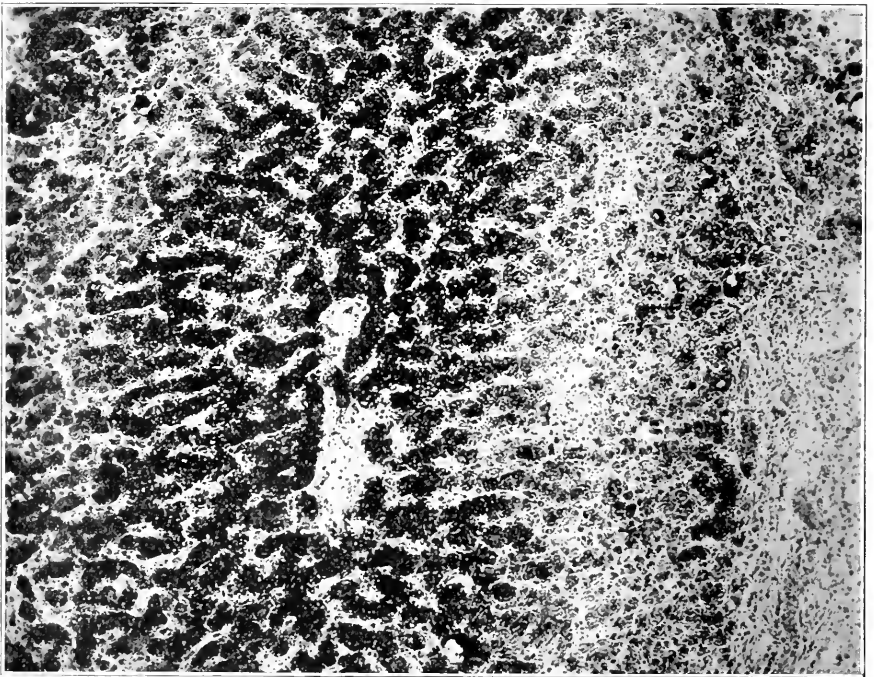


Fig. 6.—Section of liver-lobule from liver of Case II. Frozen section, Scharlach R and hemalum. Slightly higher magnification. Shows the same lipin changes, and the marked peripheral necrosis and beginning cirrhosis.

because of the fact that the greater part of the material was distributed through various fixing fluids at the time of the autopsy. The reactions are sufficiently characteristic, however, to enable us to state that the fatty changes in these cases are not those of ordinary fatty infiltration and degeneration but represent a mixed glycerin-ester and cholesterol-ester lipoidosis. Whether lecithin is present or not it is impossible to say. It is also evident that many of the liver cells contain chiefly cholesterol-esters. Further, this cholesterol lipoidosis is not confined to the liver, but occurs to a marked degree in the adrenals, in the panniculi, and to a lesser degree in scattered cells or groups of cells in other organs and tissues (spleen, aorta, kidney, etc.). In the panniculi the bulk of the lipins are probably glycerin-esters. The condition is then one of abnormal lipin metabolism

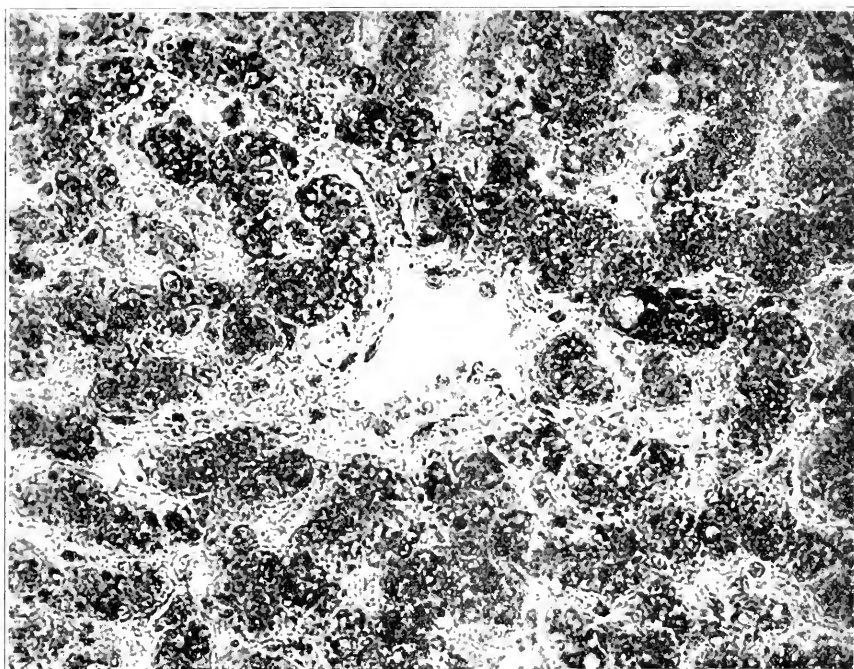


Fig. 7. Central zone of fatty globules (area C, 2). Frozen section, Schottluch R and hemalum. High power. Cells packed with small isotropic and anisotropic droplets and droves and larger isotropic deep red globules. Cholesterol- and glycerol-ester stasis.

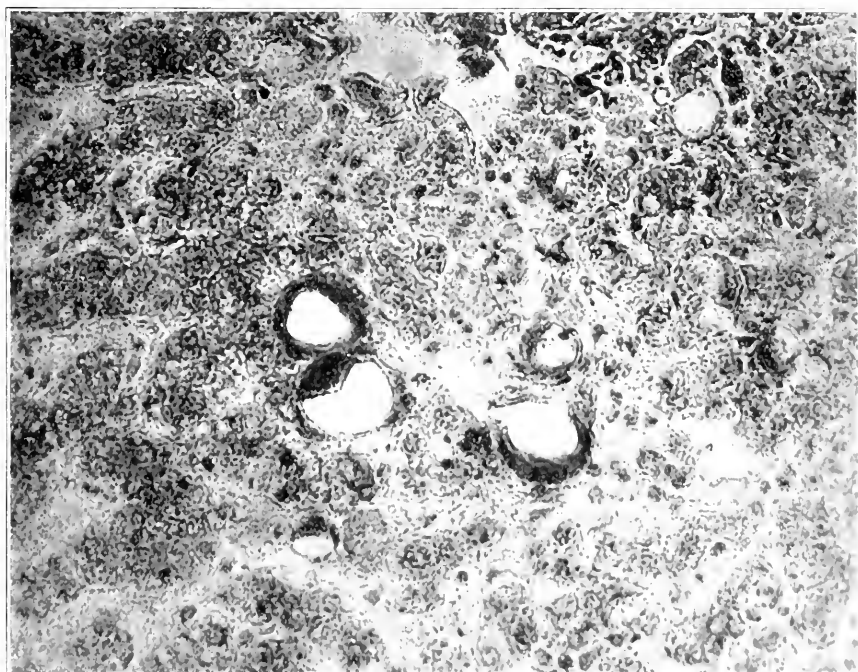


Fig. 8. Border between central zone and peripheral zone of fatty globules (area C, 2). Frozen section, Schottluch R and hemalum. High power. To the right the cells of the peripheral zone; the liver cells on the left are filled with crystals and granules of cholesterol. The peripheral zone is filled with fat partly torn out in freezing and isotropic fat globules, mainly deep red.

characterized particularly by cholesterin infiltration or retention (cholesterol steatosis). It is to be compared then to the cholesterol infiltrations obtained by the overfeeding with cholesterol, to the lipoidosis of diabetic lipoidemia and that of Gaucher's disease. It is, however, quite distinct in its histological picture from these conditions, although the differences as far as the deposit of the lipoids are concerned may be more of degree than of kind.

Just what relation the peculiar zonal necroses in the liver bear to the lipin metabolism, it is impossible to say. The peculiar position of this zone, intra-peripheral, makes this change a unique feature in the pathology of hypopituitarism. The cause of death of the liver cells is not evident, there are no thromboses of the liver capillaries at this point, no congestion, no cause of local asphyxia, etc. That the necrosis is not altogether recent is shown by the fibroblastic proliferation and the cellular infiltration. It is, in fact, an early cirrhosis beginning, not at the periphery of the lobule, but a little distance within it, leaving outside of it a narrow zone of unaffected liver cells. In the necrotic zone all stages of atrophy and necrosis of the liver-cells can be seen. At the borders the cytoplasm of the liver cell is filled with fat-granules; these change to brownish granules, which, in turn, as the cells become smaller, become less and less visible, until only pale colorless cell bodies remain. The best explanation of this peculiar zonal necrosis is on the assumption of some toxic substance brought in the blood stream and acting upon the liver cells at this point; or else, as a result of metabolic disturbances confined to this zone of cells.

I have searched in vain through the literature for observations of a similar intraperipheral zonal necrosis, but have found nothing comparable to this form. The mid-zonal necroses associated with marked fatty infiltration of the central zone with hyperemia and necrosis of the intermediate zone are not like this; nor does it resemble the mid-zonal necrosis seen in yellow fever. There is no hyperemia, the capillaries toward the central vein are not obstructed, and the cells of the central zone, while filled with fat droplets, are not larger than normal liver cells. Finally, the necrosis is more peripheral than mid-zonal, involving the inner portion of the peripheral zone.

Both cases showed a very striking postmortem increase in temperature. Thermometer readings were taken of the second case. Seven hours after death, with all of the internal organs removed, lying on a metal table over which cold water was running, the panniculus in the flaps of the abdominal wall still were unpleasantly warm to the touch, and showed a temperature of  $104^{\circ}$ . A large slice of the abdominal wall showed the same temperature some time after removal from the body. A clinical thermometer plunged into the liver gave a reading of  $108^{\circ}$ . It is most probable that this excessive heat-production is related to the abnormal fat deposits, and to postmortem changes in the lipins of the cell.

#### SUMMARY.

In hypopituitarism there is a peculiar obesity due to infiltration of various cells of the body with a mixture of lipins:—glycerin-esters and cholesterol-esters. This condition of cholesterol liposis is especially marked in the liver and adrenals, but is scattered all over the body. With deficiency of hypophysis



function there appears to be associated a cholesterol retention or infiltration. Hypopituitarism must, therefore, be classed among the xanthelasmic conditions, and is related in kind to diabetic liposis and Gaucher's disease.

The hypophysis is either directly or indirectly concerned with lipin metabolism, particularly with cholesterol steatosis. Postmortem hyperpyrexia may be associated with hypopituitarism and the obesity resulting from the latter condition.

In the livers of two cases of hypopituitarism there occurred a peculiar intraperipheral zonal necrosis, unlike all previously described forms of zonal liver necrosis. Associated with this necrosis is a reparative fibroblastic proliferation, giving rise to the picture of an early intralobular cirrhosis. The relationship of this hepatic change to the hypophysis conditions remains to be shown.

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## THE EMPLOYMENT OF CLOSED ETHER ANESTHESIA FOR ORDINARY LABORATORY EXPERIMENTS\*

BY D. E. JACKSON, PH.D., M.D., ST. LOUIS.

THE maintenance of a regular and reliable ether anesthesia in experimental animals is a matter of much concern to all investigators who find it necessary to carry out this form of work. For student experiments also the needs in this direction are obvious. A simple and cheap method for this purpose, requiring only such apparatus as can readily be secured in practically all laboratories for experimental pharmacology, surgery, or physiology is described in the following paragraphs.

Fig. 1 shows the arrangement of the apparatus complete as it is adjusted to an animal in which a cannula has been inserted into the trachea. The large pan should be about nine and one-half inches in diameter for animals (cats, rabbits) ranging in size up to dogs weighing about 15 to 20 pounds. The depth of the pan should be about three inches. For this purpose I have used a large, round, tinned iron (not aluminum) cake-pan purchased at a ten cent store. For large dogs the pan should be somewhat larger than the one here described. On pans of this type the upper edge is turned outward thus forming a kind of flange with a rounded edge. At a distance of about one-half inch below this a second ring (or flange) must be soldered around the pan. This ring should extend out from the wall of the pan about one-fourth of an inch. The ring may be made of a curved strip of tinned iron, or brass or copper, or a one-fourth inch soft copper tube may be bent around the pan and soldered on. (The copper tube will cost about 45 cents.)

Fig. 2 shows a plan of the pan as seen from above. Into the left hand side of the pan a brass spout made of one and one-eighth inch brass tubing is soldered. This tube enters the pan about half way down its side and extends upward as it passes outward from the pan. At a distance of about two inches from the wall of the pan this tube has an obtuse bend which allows the end of the spout to pass outward in a horizontal direction. This greatly facilitates its attachment to a tracheal cannula or to an air-tight muzzle (Figs. 3 and 4), if the windpipe is not opened. It is important for this tube to be *short* and of comparatively large bore, as this produces the least possible obstruction to the breathing of the animal. A large perforated cork is placed in the horizontal portion of the spout and the side tube of the tracheal cannula is passed tightly through the cork. The free end of the tracheal cannula carries a short piece of rubber tubing which is closed off as the cannula is inserted into the trachea and then attached to the spout of the pan. If a muzzle is used, one of the projecting flanges either at the end or on the side of the muzzle (depending on the position of the animal) is slipped over the horizontal part of the spout on the pan and the connection is made air tight by means of a broad rubber band covering the joint where the tubes overlap. The other flange on the muzzle is then closed with a stopper.

\*From the Department of Pharmacology of Washington University Medical School.



From the right-hand side of the pan a brass (oxygen inlet) tube one-fourth inch in diameter passes into the centre of the large pan. At this point

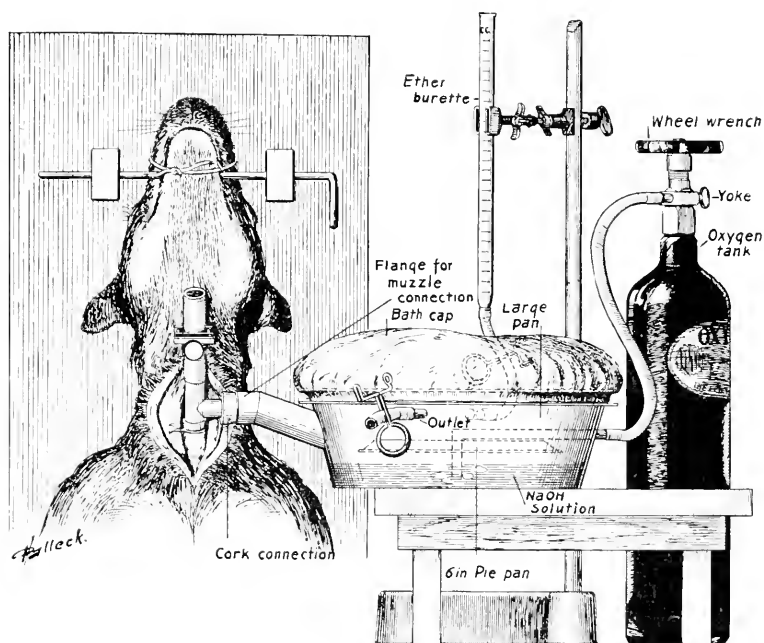


Fig. 1.—A diagram showing the general appearance and arrangement of the apparatus as attached to the side tube of a tracheal cannula. Oxygen is admitted slowly from the tank. The large pan is placed on a small table close to the operating board. The dog and the operating board are shown diagrammatically as if placed in an upright position.

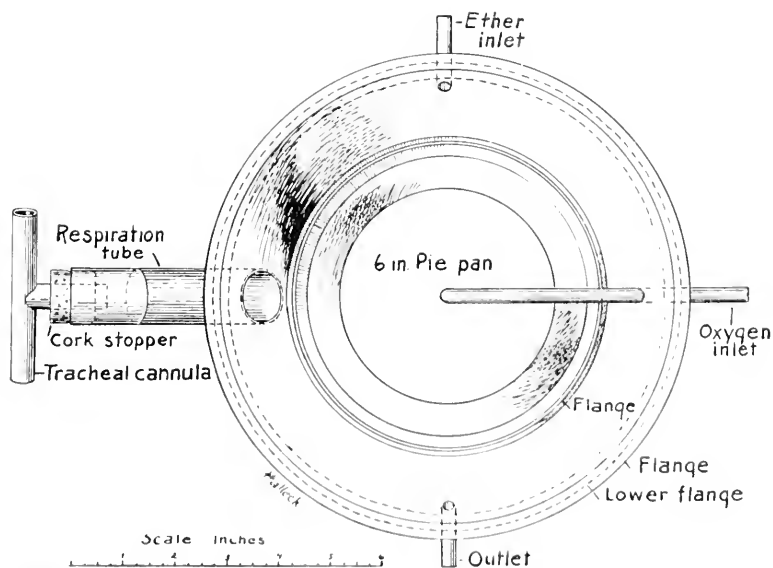


Fig. 2.—A general plan of the apparatus (without the bath cap) as seen from above.

the tube bends downward at a right angle and ends about one-fourth inch from the bottom of the large pan. This tube carries a small (six inch) pie pan (two

can be bought for 5 cents at a ten cent store) which is attached, bottom upwards, just below the angle in the brass tube. The perpendicular portion of the tube passes through a hole made in the centre of the bottom of the small pan. The outer flange of the small pan should be placed at a distance of about one and one-eighth inches above the bottom of the large pan.

Two other brass tubes one-fourth inch in diameter and one inch long are soldered into the upper part of the opposite sides of the large pan just below the lower flange (or ring). One of these tubes serves as an inlet for ether which is admitted from a burette through a rubber tube, while the other brass tube is used as an outlet and carries a short piece of rubber tubing closed by a pinch cock.

When in use a very thin rubber bath cap is stretched over the large pan in such a manner as to allow the rim of the cap to close in between the upper edge (flange) of the pan and the lower flange (ring). This connection must be air tight, but this is practically always accomplished at the first trial if wrinkles are kept out of the cap band. There are many varieties of these caps on the market. The one I have found most satisfactory is made of two pieces of rubber, a round sheet about ten and one-half inches in diameter which serves as the top of the cap, and a second round sheet of approximately the same diameter but having a circular opening about four and one-half inches in diameter cut out of its centre. This opening is the portion which is stretched out to fit over the pan. These two sheets of rubber are cemented together at the outer edges and no puckering or wrinkling of the edges of the cap occurs when the rim is fitted down over the pan. These caps are made of very thin yellow rubber and cost 25 cents apiece (at a large department store). Higher priced caps are to be avoided as they are likely to be made of heavier material, and often have folded edges (which cause leaks of air around the rim of the pan). If reasonable care is taken of one of these caps it will last for many weeks. It should be washed and dried and preferably dusted with powdered starch each time after use.

For use the pan has a layer of strong (not quite saturated) sodium hydrate solution (calcium hydrate may be added if desired) about three-fourths of an inch in depth poured into the bottom. The oxygen inlet tube extends below this solution and when oxygen is admitted from the tank the solution is splashed upward against the small pan. This serves to help the sodium hydrate to absorb the carbon dioxid exhaled by the animal. The small pan prevents the solution from splashing up into the spout and reaching the lungs of the animal. Ether is admitted from a burette and only very small quantities are needed. Injections from the burette may often not exceed one-half or one cubic centimeter for half an hour after the animal is once thoroughly well anesthetized. Some ether usually has to be added from time to time, but the greatest danger in the use of the apparatus is the giving of too much ether. This is well shown in Fig. 5, in which one cubic centimeter of ether was injected when the animal was already fairly deeply anesthetized. This ether was later allowed to escape (by opening the screw clamp at the end of the tracheal cannula).

If a muzzle similar to the one shown in Figs. 3 and 4 is used the animal may be attached to the operating board and anesthetized with ether injected

into the pan. I have, however, usually anesthetized the animal on the floor and then inserted a tracheal cannula which is at once attached to the pan. This method is more rapid but more ether may be wasted in starting the anesthesia.

Regarding the cost of anesthesia for animals by this method I may give the following data which is only an approximation of the expense which varies greatly with the size (species) of the animal and with the nature of the experiment. In a series of ten experiments on dogs (some very large, others

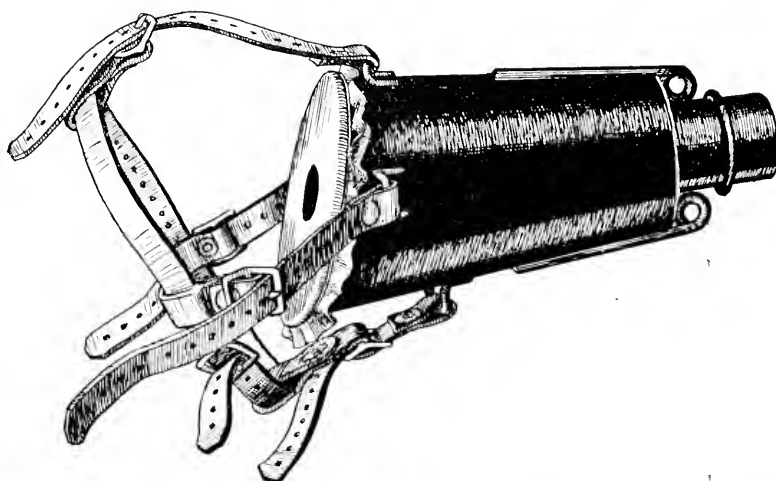


Fig. 3.—An air-tight muzzle as used to administer an anesthetic to a dog if the trachea is not opened. The rear end of the metal (brass) cylinder is covered with a disc of heavy rubber dam which is perforated with a three-fourths inch hole near the center. The dog's nose and mouth are passed into the muzzle through this hole.

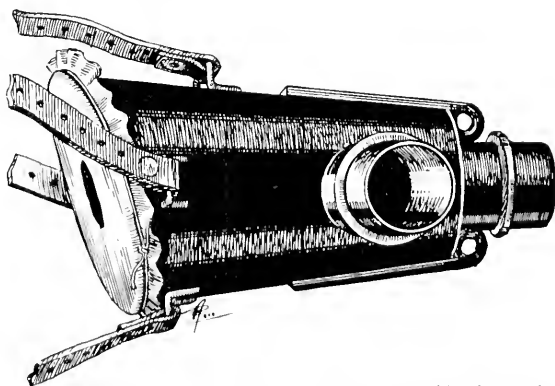


Fig. 4.—A view of the left side of the muzzle showing the side flange for use when the animal is lying on its back. The flange slips over the spout of the large pan, the connection being covered with a broad rubber band.

small) in which the anesthesia lasted from perhaps one hour up to seven hours and ten minutes I used one forty gallon tank full of oxygen, approximately two pounds of sodium hydrate and about one pound of ether. The oxygen (in 40 gallon tanks, one of the most expensive sizes) cost \$2.25, the sodium hydrate cost \$0.50 per pound (abnormally expensive) and the ether \$0.36 per pound. This indicates that the cost of these experiments was about \$0.36 per animal, and as the average duration of each experiment was approximately four hours

the average cost per hour was about nine or ten cents. This must necessarily vary greatly, however, with the size of the animal, for a small dog will use very much less oxygen and sodium hydrate than a large one. The cost of the

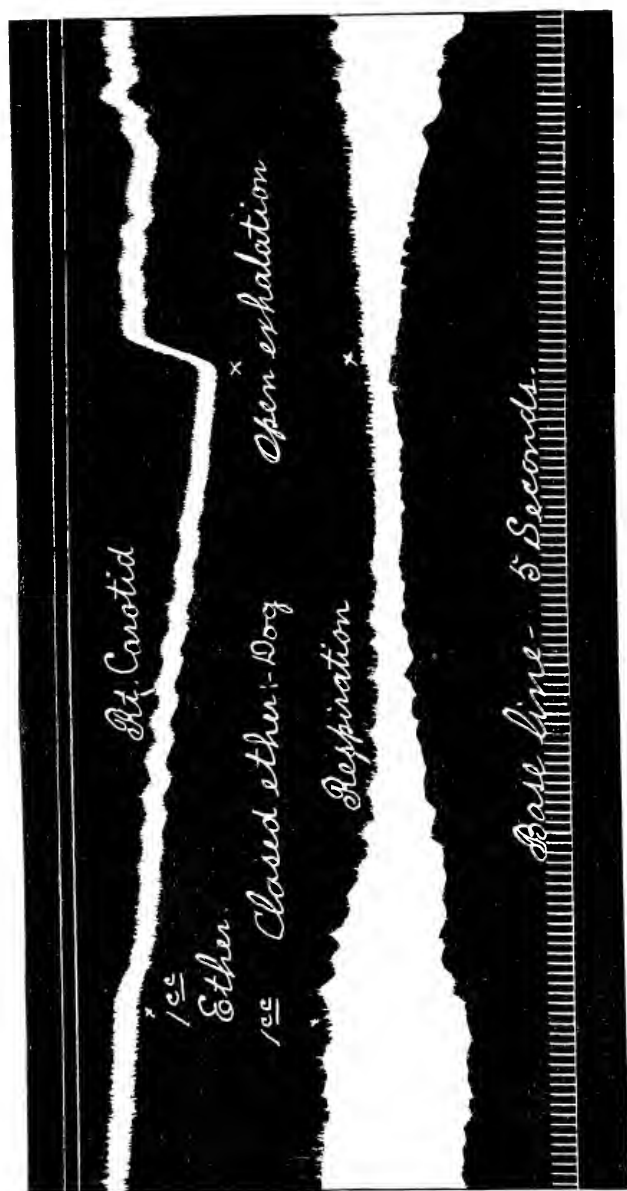


Fig. 5.—Blood pressure (upper) and respiration (lower) tracings from a dog. The animal was fairly deeply anesthetized and had received no ether for some time. At the point marked by the first two crosses 1 cubic centimeter of ether was injected into the apparatus from the burette. The effect of this comes on gradually and progressively until at the second set of crosses the tracheal cannula was opened to the outside and the animal allowed to exhale the excess ether. Recovery then rapidly occurred.

ether is very small in any case. The chief point to be considered in this method is, however, not the initial cost of the chemicals, but the greatly improved and reliable form of anesthesia. For in a closed method such as this, one "dose" of ether is retained with but exceedingly small variations within the animal over prolonged periods of time, and the regularity of the anesthesia compares

more with that produced by a "dose" of morphine than with the ordinary form of anesthesia carried on by the open methods, with ether bottles, etc. One has full command of the depth of the anesthesia and can vary it at any time. Chloroform, ethylchlorid or nitrous oxid may also be used by this method but for ordinary experimental work ether is preferable.

There were three points about which I was in doubt before I had carefully tried out this method. These were first, the question as to whether or not the movement of the respired air (or vapors) above the sodium hydrate solution would be sufficient to cause a satisfactory absorption of the carbon dioxide exhaled by the animal. With very large dogs I believe that  $\text{CO}_2$  may thus accumulate to some extent at times especially if the alkali solution is not sufficiently strong. With smaller animals (also rabbits, etc.) the  $\text{CO}_2$  causes no disturbance and is apparently practically completely absorbed as soon as exhaled from the lungs. With a larger form of apparatus this absorption would undoubtedly be sufficient for any animal. It was with this object in view that the wide shallow cake pan was used so as to provide for a large absorbing surface.

The second point about which I was in doubt concerned the possible accumulation of too much moisture in the air breathed. It is to be remembered in this connection that dogs give off exceedingly little moisture in the form of sweat and excretion of water by the lungs becomes of especial importance. This accumulation of watery vapor, however, depends mainly on temperature. This was the third point about which I was especially concerned. In practice it does not appear, however, that either of these factors causes any special disturbance. This is probably due to the use of a considerable volume of cold alkali solution and the further rapid cooling of the air to room temperature which can occur through the very thin and flexible bath cap. A further factor in the cooling also is the admission of oxygen from the high pressure tank. The sudden expansion of this gas as it bubbles through the hydrate solution cools very considerably the oxygen admitted. Unless the dog be too large for the capacity of the apparatus none of these factors causes any objectionable features in the anesthesia.

It is important for the alkali solution to be cold, however, when it is used. The alkali should be dissolved and the solution allowed to cool for several hours before it is employed for an experiment. In one instance, by mistake, some sticks of sodium hydrate were added to the solution (which had been used before) in order to strengthen it just before an experiment was started. These fresh sticks in dissolving raised the temperature of the alkali solution in the large pan considerably. Correspondingly it was found that the rectal temperature of the animal rose to 105 degrees Fahrenheit. In average experiments, however, the temperature usually remains practically constant at the point registered when the apparatus is attached to the animal, but in long experiments, or following severe operations, the temperature slowly falls. It has seemed to me that the temperature often thus remains up to normal thereby conserving the vitality of the animal at times when ordinary open methods of anesthesia would considerably weaken the animal through heat loss.

Certain interesting features are often brought out by this method of anes-

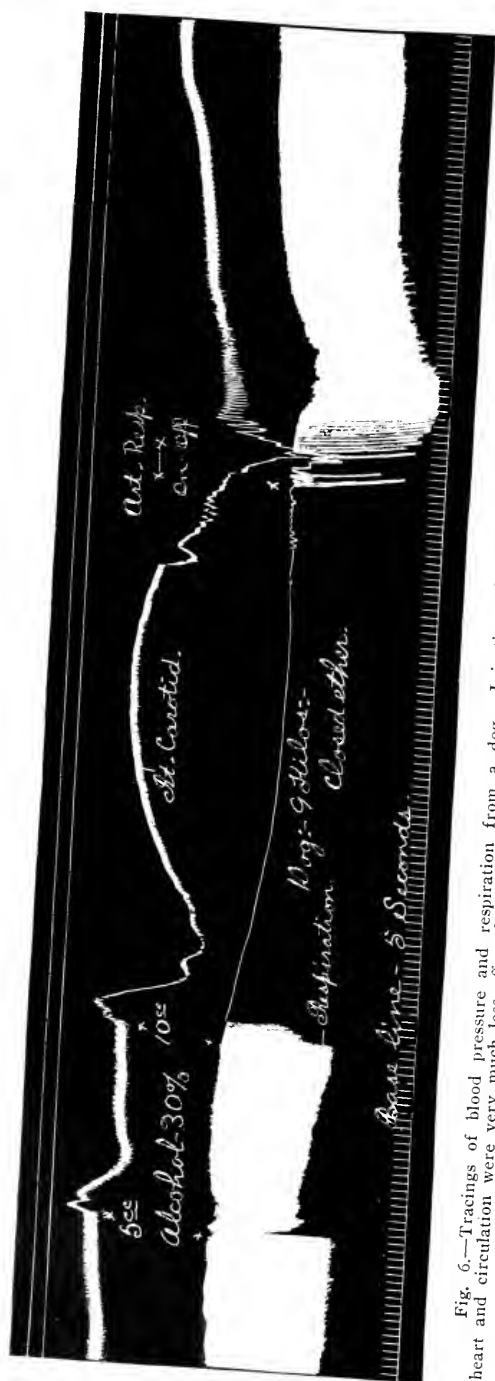


Fig. 6.—Tracings of blood pressure and respiration from a dog. Injection of ethyl alcohol as indicated stopped the respiration but the heart and circulation were very much less affected probably because of the abundant supply of almost pure oxygen.

thetia. One of these is illustrated in Fig. 6 which shows the action of alcohol on the circulation and respiration. The second injection (intravenously) of ten cubic centimeters of 30% alcohol completely stopped the respiration. There was a considerable initial fall of blood pressure followed by a rise

(asphyxial?), but the pressure did not again soon begin to fall rapidly as would ordinarily occur. On the contrary the pressure remained high for a very considerable period of time during which the respiratory movements were entirely absent. Finally, as the blood pressure started to fall rapidly, the lungs were inflated a few times artificially and then the animal at once started rapidly to

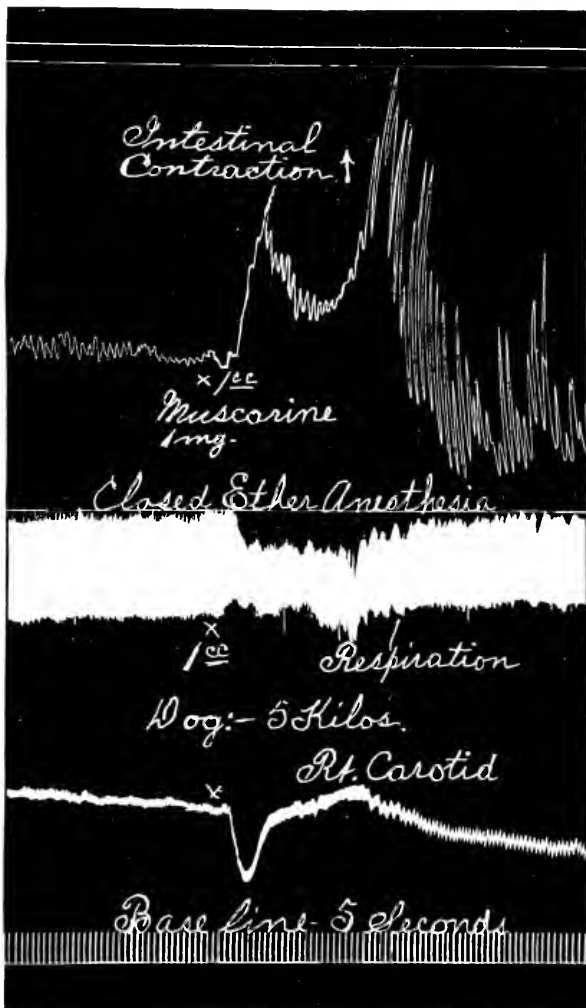


Fig. 7.—Intestinal contractions, respiration and blood pressure of a dog under closed ether anesthesia. Injection of 1 milligram of muscarine caused marked reactions as indicated in the tracings.

recover. The point of interest here is the very long period during which the animal lived in very good condition while the respiration was stopped. It would appear that the approximately pure oxygen in the apparatus, with which the lungs were in fairly direct communication through the trachea, must have been in part responsible for the result observed.

Another point of interest is the fact that, as a rule, one must from time to time add small amounts of ether to the apparatus to maintain the anesthesia

at a constant depth. It would seem that this may be due to the very gradual destruction of small quantities of the drug or to its excretion in traces from the tissues of the animal. I have suspected that a very limited amount may thus escape through the mucous membranes of the mouth. Other small quantities may be excreted into the urine or pass into the intestinal contents or into the structure of the bones, etc., when the depression of the central nervous system might be decreased a little thus allowing slight symptoms of recovery to appear. It is also possible that small traces of the drug may leak out through the rubber bath cap. In all cases, however, it is strikingly evident that only the smallest amounts of ether can either be oxidized or excreted during the course of the longest anesthesia. When the breath is allowed to escape into the open air the ether is of course rapidly excreted by the lungs.



## DIAGNOSIS OF CANCER\*

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THE early diagnosis of cancer is so important for the welfare of the patient, that clinical methods alone are only of limited and usually precarious significance. The laboratory has been striving for a number of years to develop a test for cancer which should be as reliable as the Wassermann test is for syphilis.

In the past two decades or so, quite a number of methods have been suggested for the early diagnosis of malignant neoplasms. Not one of the methods thus far recommended has proved specific and infallible. The enthusiastic claims of the originator of each laboratory procedure could not be corroborated by the various other workers in this field. Nevertheless, a combination of several methods may aid the clinician very much in the early diagnosis of cancer. The tests thus far suggested may be classified as follows:\*\*

### A. Microscopical.

1. Examination of shreds of pieces of tissue.
2. Examination for bacteria.
3. Examination for protozoa, etc.

### B. Biological.

#### 1. Hematological.

- a. Analysis for occult blood.
- b. Presence of specific bodies in blood.
- c. Serological.
  - i. Deviation of the complement.
  - ii. Anaphylactic studies.
  - iii. Presence of precipitins, lysins, hemolysins, etc.
  - iv. Presence of antitrypsin.
  - v. Toxicity of serum.
  - vi. The Abderhalden reaction.
  - vii. The Meistagmin reaction.

#### 2. Biochemical.

- a. Gastric contents' analyses.
  - i. The glycol-tryptophane and other polypeptid reactions.
  - ii. The Salomon albumin test.
  - iii. Anaphylactic studies.
  - iv. Enzyme tests.
- b. Urinary analyses.
  - i. Enzyme tests.
  - ii. Metabolism studies of nitrogen, sulfur and phosphorus.

### C. Clinical methods.

1. Test diets—Glutzinski, etc.
2. Skin reactions and vaccine methods.

### D. Chemical.

1. Analyses of gastric contents.
  - a. Presence of lactic, acetic, oleic acids, etc.
  - b. The hypoacidity determinations.
  - c. Tests for blood.
  - d. Analysis for nitrogen, albumin, phosphorus etc.

\*From the Biochemical Laboratory, Western Pennsylvania Hospital, Pittsburgh, Pa.

\*\*Kahn, Max: Arch. Diagnosis, July, 1914.

2. Analysis of urine.
  - a. Colloidal nitrogen estimation.
  - b. Neutral sulfur determination.
  - c. Determination of oxyproteic acid.
  - d. Sundry other reactions.
3. Examination of puncture fluids from serous cavities.

We have compiled in a table of statistics the findings of various scientists, who have reported either favorable or unfavorable results with the more important of these tests.

TESTS.	FAVORABLE				NON-FAVORABLE			
	CANCER		NON-CANCER		CANCER		NON-CANCER	
	%	%	%	%	%	%	%	%
	+	-	+	-	+	-	+	-
The Abderhalden Reaction	100	0	6	94	30	70	10	90
The Meistagmin	89	11	0.9	94.1	83	7	40	60
The von Dungern	89.8	10.2	7.2	92.8	40	60	35	65
The Brieger-Trebing	93.6	6.4	30.7	69.3	56	44	75	25
The Salomon Test	95	5	10	90	60	40	10	90
The Salkowski-Kojo Test	93	7	12	88	70	30	20	80
The Salomon-Saxl Test	85	15	7	93	80	20	45	55
The Kelling Test	81	19	18	82	70	30	35	65
Glycyl-Tryptophane	95	5	0	100	95	5	90	10
Oxy-proteic Acid	90	10	8	92	80	20	40	60

We have given special attention to the Salomon test for gastric cancer, and to the Salkowski-Kojo and the Salomon-Saxl test for malignancy of any part of the body.

#### I. SALOMON TEST FOR GASTRIC CANCER.\*

On the assumption that malignant disease of the stomach is accompanied by degeneration processes which liberate debris and protein matter into the stomach cavity, Salomon recommended a test which he thought was diagnostic of cancer of the stomach. This theoretical assumption is in accordance with our knowledge of cancer in general, and the results reported by various observers would seem to bear out the theory. It must be remarked, however, that such diseases as gastric ulcer would also cause a gastroalbumorrhea, as has been demonstrated by several opponents of Salomon's test.

Salomon's method for testing the stomach contents for the albumin fraction is as follows: The stomach is first carefully washed on the evening before testing, after a preliminary nonalbuminous fluid diet had been administered for twenty-four hours. On the next morning the stomach is thoroughly washed with normal saline solution (400 c.c.) the same fluid being repeatedly used and then tested for the quantity of nitrogen by the Kjeldahl method and for the quantity of albumin by the Esbach method.

Salomon found the nitrogen content in noncarcinomatous cases to be between 0 and 16 milligrams per 100 c.c. of fluid. His study of six cases of cancer of the stomach revealed between 10 and 70 milligrams of nitrogen per c.c., and the albumin content was between 0.06 and 0.5 parts per thousand. According to Salomon a case is extremely suspicious of carcinoma if the nitrogen con-

\*Kahn, Max, and Jacobowitz, I.: Med. Rec., New York, Dec. 11, 1915.

ent is more than 20 milligrams per 100 c.c. of the fluid, or if the Esbach test gives a distinct precipitate.

Wolff and Junghans modified the Salomon technic somewhat. They determined the albumin by the phosphotungstic acid reagent. They obtained very good results. Smithies found that the Wolff-Junghans modification is of decided value. Another modification of this test has been recommended by Goodman, who desired to eliminate the labor involved in a Kjeldahl determination. He analyzed the gastric contents for phosphorus.

Siegel concurred with Salomon's opinion, concluding from his own results that a figure over 25 milligrams of nitrogen per 100 c.c. is suspicious of gastric cancer, Orłowski, Schittenhelm and Lowes, Zirkelbach, Witte, and Schupfer are convinced that the Salomon test is of value. Gerster regards this test as useful in cancer of the lesser curvature without stenosis, unless the cancer has formed in an old ulcer, in which case the little hydrochloric acid present would digest the albumin present. Zirkelbach, however, is of the opinion that the minimum nitrogen content suggestive of cancer is 30 milligrams nitrogen per 100 c.c. of the washing fluid. Berent and Guttmann, Romano, Minkowski and Yague have reported very unfavorable results with this test. Goodman, on the basis of his findings with his modified technic, concluded that: (1) In normal individuals and in persons suffering from diseases exclusive of carcinoma of the stomach, the Salomon test gives more than 20 milligrams of nitrogen per 100 c.c. of wash water; (2) not all cases of gastric carcinoma reveal more than 20 milligrams of nitrogen—the absence of ulceration is probably responsible for this; (3) the test is by no means pathognomonic and can be considered as contributory only to the other symptoms; (4) the phosphoric acid of the wash water of a noncarcinomatous case is less than 10 milligrams per 100 c.c., whereas in cancerous conditions it usually exceeds 10 milligrams.

Mention should be made of the work of Clarke and Rehfuss, which was communicated by Hawk before the Columbia University Biochemical Association. The following is a summary of their findings: "The protein content of the gastric juice was investigated by the method of Wolff, namely, by successively diluting the gastric juice and adding phosphotungstic reagent. It was found that the normal gastric juice *per se* contained only traces of protein, never giving a reaction in dilutions greater than 1:40. The protein content of the specimens removed by the fractional method, after administration of Ewald meals, was also determined. The macerated Ewald meal *in vitro* never gave a reaction in a dilution greater than 1:40. Treated in the incubator with artificial gastric juice, the authors were unable to demonstrate an increasing content due to the effect of the gastric juice on the proteins of the bread. If, therefore, material removed from the stomach at intervals develops a greater protein content than the theoretical content due to the action of the gastric juice on the bread proteins, we are able to say that it comes from other sources than bread." In cancer the authors found a high protein coefficient.

We have found the Salomon test to be of distinct value in the diagnosis of cancer of the stomach. It will be seen from the accompanying tables that the figures obtained in gastric malignancy were very much higher than the figures

## ALBUMIN IN THE GASTRIC WASHINGS OF NONGASTRIC CASES.

NO.	NAME.	DIAGNOSIS.	NITROGEN MGS. IN 100 C.C.	ALBUMIN PARTS P.M.	
				ESBACH.	TSUCHIYA.
1	J. M.	Diabetes	12.5	Traces	Traces
2	I. B.	Diabetes	9.4	None	Traces
3	N. R.	Pulm. Tbc.	7.3	None	None
4	F. M.	Fracture	5.9	None	Traces
5	A. J.	Fracture	1.4	None	None
6	S. H.	Syphilis	8.2	None	None
7	A. K.	Syphilis	5.4	None	None

## GASTROALBUMORRHEA IN CARCINOMA OF THE STOMACH.

NO.	NAME.	NITROGEN MGS. IN 100 C.C.	ALBUMIN PARTS P.M.	
			ESBACH.	TSUCHIYA.
8	Mrs. W.	69.8	0.75	0.8
9	M. W.	52.7	0.6	0.75
10	F. C.	71.5	0.65	0.8
11	S. G.	47.9	0.6	0.8
12	E. F.	33.2	0.4	0.5
13	M. G.	44.8	0.5	0.7
14	Z. H.	51.2	0.5	0.6
15	A. T.	32.4	0.4	0.5
16	D. S.	67.4	0.75	0.9
17	E. M.	62.8	0.8	0.9
18	W. J.	32.5	0.6	0.8
19	A. H.	27.9	0.4	0.6
20	G. T.	35.5	0.5	0.75
21	G. F.	29.3	0.4	0.75
22	J. L.	51.2	0.5	0.6

## IN CANCER OF THE ESOPHAGUS.

23	N. Z.	62.7	1.2	1.5
24	N. H.	28.4	0.4	0.6
25	J. T.	43.9	0.6	0.8

## GASTROALBUMORRHEA IN NONCANCEROUS DISEASES OF THE STOMACH.

NO.	NAME.	DIAGNOSIS.	NITROGEN. MGS. IN 100 C.C.	ALBUMIN PARTS P.M.	
				ESBACH.	TSUCHIYA.
26	B.	Gastric ulcer	25.5	0.2	0.2
27	S. K.	Gastric ulcer	32.4	0.4	0.4
28	M. F.	Gastric ulcer	41.2	0.5	0.6
29	M. M.	Gastric ulcer	33.7	0.4	0.3
30	B. W.	Gastric ulcer	35.9	0.4	0.5
31	J. J.	Gastric ulcer	22.6	0.3	0.3
32	R. K.	Gastric ulcer	12.8	None	Trace
33	K.	Cardiospasm	1.5	None	None
34	M.	Acute gastritis	38.9	0.5	0.5
35	F.	Hyperacidity	12.5	None	Traces
36	Katz	Gastrectasis	3.9	None	None
37	L. B.	Tabetic crises	2.4	None	None
38	T.	Hyperacidity	1.7	None	None
39	L. K.	Chronic gastritis	19.8	0.2	0.3
40	J. U.	Anacidity	5.5	None	Trace
41	M. D.	Appendicitis	2.6	None	None
42	S.	Hernia	2.4	None	None
43	M. M.	Intestinal stasis	4.7	None	None

found in other diseases of the stomach. Care must be observed to eliminate gastric ulcer and acute inflammation of the stomach mucosa. A negative Salomon test is significant of a nonmalignant condition. A positive test has to be judged discriminatingly.

We wish to call attention to the fact that ordinary, routine examination of the gastric contents after an Ewald test meal is of but little value as an aid to diagnosis of gastric carcinoma.

Graham and Guthrie analyzed the gastric contents of 150 patients suffering with carcinoma ventriculi. They obtained the following results, which we shall present in the form of a table:

Free hydrochloric acid present in.....	70 cases
Free hydrochloric acid (no blood, lactic acid, food) in.....	46 "
Blood present in .....	80 "
Blood and lactic acid present in.....	20 "
Blood and food present in.....	15 "
Blood and food and lactic acid present in.....	30 "
Food remnants present in.....	62 "
Lactic acid present in.....	64 "

## II. THE SALKOWSKI-KOJO AND THE SALOMON-SAXL TESTS.\*

*Salkowski-Kojo Test.*—In 1892, Töpfer found that the urine of patients suffering from cancer contained a very large amount of "extractive substance." This "extractive substance" was calculated by first determining the quantity of total nitrogen and then subtracting, from this amount, the sum of the nitrogen values for urea, uric acid, and ammonia, of the same urine. Bondzynski and Gottlieb, five years later, reported that the nitrogen in oxyproteic acid, in the urine, was 2 to 3 per cent of the total urinary nitrogen. Salkowski, and Hess and Saxl, using different procedures, concluded that the oxyproteic acid portion of the alcohol-precipitable substances is increased in the urine of human beings suffering from carcinoma.

Salkowski and Kojo, in a preliminary communication, recently suggested several methods for the determination of colloidal nitrogen in the urine. A year later, Kojo published the results of a comparative study of the various procedures suggested in this connection. Kahn and Rosenbloom studied the zinc-sulfate-precipitable, colloidal, nitrogenous material from the urine of normal subjects, as well as of carcinomatous patients, and concluded that the amount of colloidal nitrogen was invariably increased in carcinoma. They also found that diseases like myocarditis, diabetes, leukemia, and anemia, likewise gave a high colloidal-nitrogen index. They concluded that this quantitative test was not specific for cancer. Kahn and Rosenbloom studied the amount of colloidal nitrogen in the urine of a dog suffering from a malignant neoplasm. In this case they used dialysis as a part of the method and found that the quantity of colloidal nitrogen was much greater in the urine of the diseased dog than the amount present in the urine of normal dogs.

Volpe found that the colloidal-nitrogen index is of special value in cancer diagnosis. Mancini, using the Salkowski method, found that there were in-

\*Goodridge, F. G., and Kahn, Max: Biochem. Bull., 1915, iv, 118.

creased eliminations of colloidal nitrogen in the urines of patients afflicted with cancer, but this increase also occurred in pneumonia and pleurisy. Seminov reported that the colloidal nitrogen output is low in normal individuals and is increased in cancer patients. He concluded that although the normal index excludes the possibility of a malignant growth, the increased amount of colloidal nitrogen in the urine is not specific for carcinoma. Konikov found that the average amount of colloidal nitrogen in the urine, as determined by the Salkowski-Kojo method, was 1.68 per cent of the total nitrogen in normal cases, and 2.47 per cent in carcinomatous individuals. Of 73 cases of cancer investigated by him, only 9 showed a higher coefficient than 2.5 per cent.

According to Marcel, Labbe, Dauphin and others, on the other hand, increase in the urinary colloidal nitrogen is an index of a derangement of nitrogenous metabolism; and while it may serve to detect functional insufficiency in the liver, it is not at all specific for cancerous states. Carforio, also, concluded that the colloidal nitrogen index is not pathognomonic of cancer.

*Salomon-Saxl Test.*—Salomon and Saxl have described a neutral-sulfur reaction in the urine. Like all other tests in this connection, it has given excellent results in some hands but, in others, has proved valueless. The abnormal constituent in the urine of carcinomatous patients is a neutral-sulfur fraction, the sulfur of which can be split off by means of hydrogen peroxide, and can be determined as barium sulfate. Positive urines yield 0.010 to 0.018 gm. of barium sulfate from this fraction, for 100 c.c. of urine. Of 41 carcinoma cases examined by Salomon and Saxl, 30 were positive, 4 faintly positive, 1 questionable, and 6 negative. Of 182 normal urines, 6 were positive, 3 faintly positive, 1 questionable and 172 negative.

Peterson divided his cases into three classes. (A) Clinically noncancerous suspects: of 26 patients examined, 25 gave a negative Salomon and Saxl neutral-sulfur reaction. (B) Clinically cancer suspects: of 20 cases examined, 5 were negative, 2 alternately positive and negative reactions, and 13 cases positive. (C) Manifest cancer: of 19 cases, 17 always gave a good positive reaction; the two negatives were icteric and cachectic. Dozzi found that the test was invariably negative in all his patients free from cancer or tuberculosis, but the frequency of the positive responses in tuberculous patients detracted from its value as a sign of cancer, although cancer is rarely mistaken for tuberculosis. The only cancer cases that gave negative results were those in which the cancer had been excised. Murachi, also, found an increase in the neutral sulfur from cancer patients. The coefficient, according to him, may be 3.8 per cent of the total sulfur.

In contrast to the foregoing, Pribram found that only 60 per cent of cancer patients gave a positive Salomon-Saxl test, that the test is, therefore, far from specific. Alekseev came to a similar conclusion. Mazzitelli has studied this test in 50 cases of cancer, with and without cachexia. Of 18 cases of the latter variety, the test was positive in 14; but also in 8 of 10 cases of tuberculous cachexia, and 16 or 23 cases of cachexia of various origins, including 11 with cancer and 4 with tuberculosis; Greenwald concluded that this test has no value in the diagnosis of cancer.

The procedure for carrying out these tests is as follows: Of course, the specimen of urine examined is a twenty-four hour collection, preserved with about 5 grams thymol.

*Salkowski-Kojo Test.*—The urine was first tested for coagulable protein, which, if found, was removed by means of heat coagulation, with addition to the boiling liquid of a few drops of dilute acetic acid sol. To 100 c.c. of mixed, filtered, 24 hour specimen of urine, zinc sulfate was added in sufficient quantity to effect saturation. The saturated liquid was allowed to stand for 24 hours, then was filtered through ashless paper, and the precipitate washed several times on the paper with saturated zinc sulfate solution, to remove nitrogenous substances adherent to the precipitate. The paper and precipitate were then placed in a Kjeldahl flask and the nitrogen content determined by the Kjeldahl method. The total nitrogen in 5 c.c. of urine was also determined by the Kjeldahl method. The ratio of the nitrogen in the zinc sulfate precipitate to the total urinary nitrogen was computed.

*Salomon-Saxl Test.*—The technic of the Salomon and Saxl neutral-sulfur test is the following: 150 c.c. of urine, freed from coagulable protein by heat and acid, are diluted with 100 c.c. of water. A mixture of 100 c.c. of sat. aqueous sol. of barium hydroxid and 50 c.c. of sat. aqueous sol. of barium chlorid is added, the liquid filtered, and the filtrate tested with barium to see if precipitation is complete. In order to remove the ethereal sulfates, 300 c.c. of the filtrate are treated with 30 c.c. of conc. hydrochloric acid sol., and boiled for 15 min. in an Erlenmeyer flask, using a funnel condenser. The flask is then placed on a water-bath for 24 hours. Of the clear filtrate, 200 c.c. are mixed with 3 c.c. of hydrogen peroxide (perhydrol-Merck), and boiled for 15 min. with a funnel condenser. After boiling, the liquid is transferred to a conical graduate, where, at the end of 6 hr., the amount of precipitate is observed. Antipyrin and creosote medications interfere, according to certain authors, with this test.

Goodridge and Kahn concluded from their observations that positive results with either the colloidal-nitrogen test (Salkowski-Kojo) or the neutral-sulfur test (Salomon-Saxl), alone, are not indicative of carcinoma. When performed conjointly on urine of the same case, however, positive results with both methods are strongly indicative of malignancy. The appended table extracted from the work of Goodridge and Kahn, is representative of the figures they obtained.

No.	Name	Diagnosis	Total N in 100 c.c. urine gm.	Colloid-N in 100 c.c. urine gm.	Per cent Colloid-N of Total N	Total S in 100 c.c. urine gm.	Salomon- Saxl neu- tral S in 100 c.c. urine gm.	Per cent Neutral S in Total S
1	A. I.	Normal	0.7459	0.01006	1.35	0.112	0.0019	1.72
2	A. I.	"	0.7875	0.0098	1.25	0.109	0.0018	1.65
3	J. S.	"	0.8132	0.0109	1.81	0.097	not w'g'd	less than 1
4	M. K.	"	0.7986	0.0167	2.10	0.124	0.0027	2.07
5	D. F.	"	0.9178	0.0161	1.71	0.171	0.0033	1.94

No.	Name	Diagnosis	Total N in 100 c.c. urine gm.	Colloid-N in 100 c.c. urine gm.	Per cent Colloid-N of Total N	Total S in 100 c.c. urine gm.	Salomon- Saxl neu- tral S in 100 c.c. urine gm.	Per cent Neutral S in Total S
23	T. A.	Cancer of uterus	0.9756	0.0419	4.3	0.085	0.0031	3.7
24	M. W.	Gastric cancer	1.1071	0.0636	5.75	0.087	0.0035	4.1
25	F. C.	Gastric cancer	1.1950	0.0652	4.62	0.108	0.0041	3.8
26	A. R.	Cancer of breast	1.2104	0.0568	4.7	0.152	0.0045	2.9
27	S. G.	Gastric cancer	0.9260	0.0361	3.9	0.095	not w'g'd	less than 1
28	T. S.	Cancer of liver	0.5762	0.0247	4.3	0.104	0.0035	3.4
29	T. A.	Cancer of uterus	1.3550	0.0469	4.2	0.087	0.0032	3.7
30	M. W.	Gastric cancer	0.8722	0.0305	3.5	0.1055	0.0025	2.5
31	S. G.	Gastric cancer	0.9128	0.0447	4.9	0.1243	0.0045	4.4
32	A. R.	Cancer of breast	1.0424	0.0458	4.4	0.1175	0.0037	3.2
33	A. G.	Cancer of rectum	0.4728	0.0212	4.5	0.1480	0.0050	3.4
34	C. J.	Cancer of cervix	1.1307	0.0431	4.7	0.0875	0.0030	3.5
35	W. J.	Gastric cancer	0.9246	0.0388	4.2	0.0986	0.0036	3.7
36	B. M.	Cancer of liver	1.1108	0.0377	3.4	0.097	0.0031	3.2
37	K. B.	Cancer of liver	0.8229	0.0427	5.2	0.1452	0.0062	4.3
38	E. F.	Cancer of stomach	1.1055	0.0608	5.5	0.1445	0.0059	4.1
39	B. J.	Cancer of pancreas	1.1782	0.0494	4.2	0.1378	0.0060	4.4
40	E. M.	Cancer of stomach	1.1363	0.0441	3.8	0.1025	0.0043	4.2
41	B. M.	Cancer of liver	0.8912	0.0427	4.8	0.1644	0.0070	4.0
42	C. J.	Cancer of cervix	0.7755	0.0334	4.3	0.1552	0.0067	4.5
43	P. B.	Cancer of uterus	0.5737	0.0252	4.4	0.1275	0.0049	4.1
44	M. G.	Cancer of stomach	0.9345	0.0345	3.7	0.09865	0.0035	3.4
45	E. F.	Cancer of stomach	0.8548	0.0435	5.1	0.1143	0.0038	3.5
46	M. W.	Gastric cancer	0.9845	0.0502	5.1	0.0975	0.0026	2.7
47	F. O.	Cancer of pelvis	0.9642	0.0424	4.4	0.0956	0.0039	4.2
48	M. F.	Cancer of cervix	0.8750	0.0411	4.7	0.1140	0.0031	2.9
49	C. J.	Cancer of cervix	0.6437	0.0270	4.2	0.1231	0.0037	3.1
50	R. W.	Cancer of rectum	0.8821	0.0379	4.3	0.1242	0.0041	3.4
82	L. S.	Lung tbc.	0.872	0.0117	1.35	0.1409	0.0047	3.4
83	A. H.	Nephritis	0.695	0.0118	1.75	.....	not w'g'd	less than 1
86	C. H.	Myocarditis	1.078	0.0363	3.4	.....	" "	" " "
89	S. E.	Typhoid	0.9465	0.0118	1.25	.....	" "	" " "
92	B. R.	Empyema	0.8253	0.0139	1.75	.....	" "	" " "
95	H. W.	Endarteritis obliter.	0.7321	0.0102	1.4	.....	" "	" " "
97	M. R.	Endarteritis obliter.	1.0878	0.0097	0.9	.....	" "	" " "
98	J. R.	Sarcoma of leg	1.046	0.0468	4.5	.....	" "	" " "
99	C. S.	Leukemia	1.0975	0.0239	2.2	.....	" "	" " "
100	Child	Hemophilia	0.784	0.0109	1.4	0.1482	0.0033	2.4
101	C. F.	Pernicious anemia	1.095	0.0130	1.2	0.1843	0.0045	2.5
102	R. K.	Atrophic cirrhosis	0.5965	0.0106	1.8	0.1077	0.0029	2.8
104	A. E.	Pneumonia	0.6546	0.0111	1.7	.....	not w'g'd	less than 1
105	R. E.	Pneumonia	1.0725	0.0161	1.5	.....	" "	" " "
108	J. M.	Diabetes	1.0953	0.0466	4.25	.....	" "	" " "
109	J. A.	Diabetes	1.2075	0.0465	3.75	.....	" "	" " "
110	B. S.	Diabetes	0.9642	0.0501	5.2	.....	" "	" " "
113	A. K.	Syphilis	0.7114	0.0263	3.7	.....	" "	" " "
114	H. H.	Syphilis	0.7227	0.0296	4.1	.....	" "	" " "
115	S. H.	Syphilis	0.5835	0.0222	3.8	.....	" "	" " "
116	M. F.	Gastric ulcer	0.6444	0.0077	1.2	.....	" "	" " "
117	M. M.	Gastric ulcer	0.9007	0.0126	1.4	0.1586	0.0036	2.4
118	B. W.	Gastric ulcer	0.8767	0.0075	0.85	0.1755	0.0042	2.5
119	C. Z.	Gastric ulcer	0.6114	0.0109	1.7	.....	not w'g'd	less than 1
124	P. B.	Endocarditis	1.0755	0.0258	2.4	.....	" "	" " "
127	H. F.	Lung tbc.	1.2005	0.0281	1.4	0.1722	0.0063	3.7
128	N. K.	Lung tbc.	0.9234	0.0139	1.5	0.1645	0.0046	2.9



Since 1914 we have had occasion to perform these tests for malignancy in a large number of cases. From our statistics we conclude that a positive result with both the Salkowski-Kojo and Salomon-Saxl Test is very suspicious of carcinoma.

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## THE UTILIZATION OF DEXTROSE IN THE ANIMAL BODY\*

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THE common practice for determining the assimilation limit of dextrose consists in giving a weighed quantity of this sugar by mouth on an empty stomach and thereafter testing the urine at intervals for its presence. When the urine is allowed to collect for twenty-four hours, the mixed specimen shows no trace of dextrose in the majority of healthy individuals after a dose of 200 gm.; after 300 gm. a somewhat higher percentage of cases develop a mild glycosuria, but frequently no trace becomes evident even after 500 grams. Beyond the last mentioned amounts, the limit of ingestion is reached, on account of nausea, etc., and it is improbable that, even if larger amounts could be tolerated, any more of the dextrose would be absorbed than with 300 or 400 grams.<sup>1</sup> It is likely that glycosuria would have been detected in the above tests had the urine been examined at frequent intervals after taking the dextrose instead of examining a mixed specimen, but even granting this to be the case, it is clear that the assimilation limit can signify practically nothing regarding the ability of the organism to metabolize carbohydrate.

These observations have an important bearing on the now well-established practice used for the detection of incipient (so-called "latent") cases of diabetes. Following the suggestions of the von Noorden school, it is usual to consider that a patient is to be thus classified if dextrose appears in the urine after alimentary ingestion of 100 gm. dextrose on an empty stomach. In the light of the above results and for many other reasons, there is considerable doubt as to the value of this test. Thus, when a solution of dextrose is given orally, its rate of absorption will depend very largely on the motility of the stomach. If this be normal, the solution will very quickly find its way past the pyloric sphincter into the intestine, where it will be rapidly absorbed. If on the other hand the pyloric sphincter should not open freely, the passage of the dextrose into the intestine may be so delayed that no more is present in this place at one time than would be the case after an ordinary diet of polysaccharide. And even after the sugar solution enters the small intestine differences in the amount of the intestinal contents with which it becomes mixed, in the extent of bacterial growth, and in the absorption process, may very materially affect the rate at which the dextrose gains entry to the blood.

Recognition of these facts has led those who have desired to determine the assimilation limit in laboratory animals to administer the dextrose intravenously. But even this refinement in technic has not, as a rule, had the effect of rendering the results of any very evident value as a criterion of the utilization of dextrose in the animal body. The reason for this unreliability of the method is mainly that the period of injection of the dextrose solution usually occupies only a few minutes, so that it causes a sudden, instead of a very gradual increase in the sugar concentration of the blood, the conditions being quite unlike those which exist during the normal absorption of dextrose from the intestine. The

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mechanism by which the body ordinarily disposes of excessive amounts of dextrose, absorbed into the portal blood, is not adjusted to operate when the systemic blood is suddenly overcharged with this substance. In the one case the dextrose is a foodstuff; in the other, because of its excessive concentration in the blood, it is more or less of a poison. Such results, in other words, merely show us how much dextrose can be added at one time to the organism without any overflow taking place into the urine, but they furnish us with no information regarding the power of the organism to utilize a constant though moderate excess of this substance. In the one case it is the "saturation limit," in the other the "utilization limit" of the organism for dextrose, that we are really considering (Blumenthal<sup>2</sup>).

Consideration of these principles has led Woodyatt, Sansum and Wilder<sup>3</sup> to undertake a thorough reinvestigation of the whole problem of the utilization or, as they prefer to call it, the tolerance of the body for dextrose. They emphasize the obvious fact that the ability of the organism to utilize dextrose "must depend on the rate at which the tissues are able to abstract it from the blood by their combined powers, to burn it, to reduce it into fat or to polymerize it into glycogen." To form any estimate of the combined effect of these processes, we must take into account not only the amount of dextrose per unit of body weight (grams per kilo.), but also the rate of injection, for "tolerance must be regarded as a velocity, not as a weight." Woodyatt and his co-workers have devised a very satisfactory apparatus by which such injections can be made at a regular, definite rate over prolonged periods of time. We have used this apparatus extensively in this laboratory, and can vouch for its perfect reliability. It is being used now in clinical practice wherever continuous injection, not only of dextrose but of any other fluid (e.g., of bicarbonate solutions in cases of acidosis) is desired.

Briefly summarized, the conclusions which Woodyatt, etc., have so far drawn from their investigations are as follows: In a normal rabbit, dog or man, 0.8-0.9 gram. of glucose per kilo. body weight and per hour can be utilized by the organism for an indefinite time without causing glycosuria. When between 0.8 and 2 grams are injected, a part of the excess appears in the urine, steadily increasing until a maximum is reached, after which the excreted fraction remains constant (at about one-tenth). If more than about 2 grams per kg. and hr. are injected, "a large percentage of all glucose in excess of the 2 grams per kg. and hr. appears in the urine, once constant conditions are established."

Being thus furnished with accurate information regarding the tolerance of the organism for dextrose, the question naturally presents itself: how is the dextrose disposed of? As indicated above, three means of disposal are available: conversion into glycogen, oxidation into carbon dioxide and water, and reduction into fat. It is commonly assumed that the operation of these processes is pretty much in the order named; that is, the excess of dextrose is first of all dealt with by conversion of the excess into glycogen, which process is soon followed, if not accompanied from the start, by an increased combustion of dextrose in the tissues and then, if there still be an excess to deal with, by the reduction of some of the carbohydrate to fat. Important investigations have

recently been published by several workers (Murlin and Kramer,<sup>4</sup> Verzáz,<sup>5</sup> etc.), regarding the burning of the excess of dextrose, but as to the mechanism involved in its polymerization into glycogen or its reduction to fat, little is as yet known.

From the point of view of diabetes, it is obviously important that we should pay particular attention to the glycogenic mechanism, for it is the first line of defense against the invasion of the tissues by an excess of dextrose, and it is presumably only after this barrier is broken through that there is danger of the whole defensive system of the body against such an excess becoming disorganized. A fundamental question regarding this first line of defense is of course concerned with its disposition; in what parts of the body is it best developed? There are theoretically two methods by which such a problem could be investigated: first, by comparing the amount of glycogen in the various parts of the body before and after the continuous administration of dextrose, and secondly, by comparing the percentage of dextrose in the blood flowing into and out of the organs. Of these, the first mentioned method has been extensively used and the results have shown that by far the largest storage of glycogen, per unit of tissue, occurs in the liver, and the next largest in the muscles, in which however the distribution is by no means uniform, constantly active muscles such as the heart and diaphragm, being especially rich in glycogen. As judged then from the concentration of glycogen which may be present, the liver would appear to be the strongest line of defense, and the position of this organ in relationship to the usual path of invasion of dextrose would indicate that it is also the most advanced.

If instead of the concentration, we consider the total storage capacity (for glycogen) of the liver as compared with that of all the muscles, we find it to be at least potentially greater in the muscles than in the liver, so that the question naturally presents itself as to the circumstances under which this muscle storage will be called into operation. It can only be the dextrose which escapes the liver and gains entry to the systemic blood that the muscles deal with; but, to what extent does this occur? Is the glycogenic mechanism of the liver so efficient that it holds back most of the absorbed dextrose until the hepatic cells become overloaded with glycogen, or does the liver only hold back some of the excess of dextrose in the portal blood and allow the rest to pass on? As judged from the results which have been published in recent years bearing on the behavior of the percentage of dextrose in the blood of the systemic circulation during the alimentary ingestion of dextrose (Böe,<sup>6</sup> etc.), it would appear that much of the dextrose passes through the liver, thus causing a post-prandial hyperglycemia and indicating that the liver barrier is much less efficient than had previously been thought to be the case.

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To throw further light on the problem, we have been engaged for some time in an investigation of the comparative dextrose-storing abilities of the liver and muscles as judged by the second of the possible methods indicated above, namely, by a comparison of the dextrose concentration in the inflowing and outflowing blood of the liver and muscles. Theoretically, such a plan of investigation would seem to be ideal in furnishing us with the information we desire,

but in practice the interpretation of the results is considerably obscured on account of the enormous volumes of blood which pass through these organs, thus making it possible that considerable quantities of dextrose might be removed from or added to them without any very definite change occurring in the percentage of dextrose in the blood. Thus, in a dog of average size, 6 kg., about 400 c.c. of blood traverses the liver in one minute. If the percentage of dextrose should fall from 0.200 in the blood of the portal vein to 0.175 in that of the hepatic veins, it would mean that in one minute 0.1 gm. of dextrose had been retained, or 6 gm. an hour, or 1 gm. per kg. per hr. That is to say, with the amount of dextrose just over the tolerance limit of 0.8 gm. per kg. and hr., and assuming that all of the absorbed dextrose was retained by the liver, there would be a difference between the percentages of dextrose in the portal and hepatic vein bloods of only 0.025, which, although well beyond the experimental error of the methods now commonly used for sugar estimation in blood, is nevertheless far from striking.

The experiments were performed on etherized dogs that had been fed for some days previously on a mixed diet of bread and flesh. The liver and muscles would therefore contain a moderate amount of glycogen. By a technic, the details of which are described elsewhere, cannulae were then placed in the branches of the portal vein, inferior vena cava, and iliac vein; and through these cannulae small measured quantities of blood (2 c.c.) were removed at frequent intervals, and the sugar percentage determined by Pearce's modification of the Lewis-Benedict method<sup>7</sup>. Another cannula was inserted into one of the smaller branches of the mesenteric vein, through which the solution of dextrose was injected. To maintain the injection at a constant rate, the cannula was connected either with a constant pressure perfusion flask or with the Woodyatt apparatus.

In order to allow as much as possible for the slight differences in blood-sugar concentration which are to be expected, even with active glycogen formation, the blood samples were removed at frequent intervals (3-10 minutes), and curves plotted, with the percentage of dextrose along the ordinates and time intervals along the abscissa. If the curves thus drawn for the portal vein and vena cava should coincide, it would indicate that no detectable glycogen formation had occurred in the liver; and similarly, if the same were the case for those of cava and iliac vein, that the muscles of the corresponding hind limb had retained none of the dextrose. Want of correspondence of the curves need not however mean that sugar retention or liberation has occurred, for the same results would be obtained when water was added to or removed from the blood. To control this source of confusion in the interpretation of the results, enumeration of the corpuscles or estimation of the hemoglobin should have been made. The already existing complexity of the experiments, however, made it impossible for us to do this.

The results will be published in detail elsewhere, but a few of the most important conclusions which may be drawn from them will be indicated in a preliminary way here. When the dextrose is injected in amounts that are below the tolerance limit of the animals (0.8 gm. per kg. and hr.), a more or less rapid increase occurs in the blood-sugar until a certain level is attained, after which the percentage remains constant. The time required for this level, or plateau,

to be reached is definitely shorter with small than with large injections; thus, the level was reached in about 25 minutes with 0.4 gm. dextrose per kg. and hr., and 35 minutes with 0.7 gm. These facts indicate that the sugar-retaining powers of the organism very quickly adjust themselves to the increased work thrown on them by the addition of dextrose to the blood. Utilization soon becomes so adapted that the dextrose is removed from the blood as quickly as it is being added, so that after the plateau has been gained, the rate of disposal of dextrose by the organism may be determined by the rate at which the injection is being made. This gives us a valuable test object on which to investigate the influence of various conditions (such as injections of acids and alkalies, drugs, stimulation of nerves, etc.) on sugar utilization. We shall return immediately to some results of this nature, but meanwhile it is important to note that comparisons of the sugar percentage in the blood of the above mentioned veins did not show any constant differences to exist, so that we cannot conclude from them whether the liver or the muscles contributes the more to the removal of the excess of dextrose, when this excess is of small magnitude.

These results prompted us to repeat the investigations using injections of dextrose that were decidedly above the utilization limit. Under such conditions it was thought that the ability of the liver and muscles to form glycogen would become stimulated to such a degree that differences in the concentration of sugar in the inflowing and outflowing blood would become apparent. Three such experiments, each occupying several hours, were performed, the rate of injection being respectively 2.4 gm., 3.0 gm. and 3.5 gm. dextrose per kg. and hour. In all three cases the blood-sugar curves rose steadily throughout the injection periods, there being no tendency, as in the previous experiments to the establishment of a plateau. In the experiments in which 2.4 and 3.5 gm. dextrose were injected, the blood-sugar curve was decidedly highest in the portal vein, next in the vena cava, and lowest in the iliac vein, the differences between them being about equal, thus indicating, as far as can be judged from such results, that the sugar-retaining powers of the liver and combined muscles of the hind limb are equal. In the experiment in which 3.0 gm. dextrose was injected, however, the curve for the blood of the iliac vein stood constantly at a higher level than that of the vena cava, which on the other hand was distinctly lower than that of the portal vein. On superficial examination such a result would seem to indicate that, instead of retaining some of the dextrose, the muscles had actually been contributing dextrose to the blood, a conclusion which it is unlikely can be correct. A much more probable cause for the anomalous result is that the blood had lost water during its passage through the muscles, and this loss of water was probably to be accounted for by the fact that considerable quantities of hydrochloric acid were injected at frequent periods along with the dextrose. Unfortunately in this experiment neither the H-ion concentration of the blood nor the number of corpuscles in it was determined, so that we cannot be certain that our explanation of the results is correct.

These observations on the effect of large injections of dextrose are of especial interest, because it has been by such experiments that several investigators have sought to throw light on the problem of the disposal of dextrose in the animal body. The most recent work of this nature is that of Kleiner,<sup>8</sup> who

found that after the injection of large amounts of dextrose (4 gm. per kg. body weight) intravenously, the blood-sugar suddenly became enormously increased and then immediately began to fall, reaching the normal again in about 90 minutes. It was found that although some of this excess is drained away in the urine, the amount must be small, since as rapid a disappearance of the excess of sugar occurred in dogs from which the kidneys had been removed as in normal animals. In another series of animals, the aorta and vena cava were tied just above the diaphragm, so as to exclude the liver from the circulation, and it was found that the injected sugar disappeared just as quickly as in intact animals. From this result Kleiner concludes that conversion by the liver of some of the excess of sugar into glycogen cannot be an important factor in the disappearance, but that absorption by the muscles must be mainly responsible. By examination of the amount of sugar in the muscles, a great increase was found, some of the absorbed dextrose being present in a polymerized state. There is, however, one serious objection to all experiments of this nature, namely, that by the sudden addition of excessively large amounts of dextrose to the blood at one time, conditions become established which can never obtain in even the severest types of experimental diabetes. Not only will the osmotic relationships between the blood and tissue fluids be entirely upset, but toxic influences dependent either directly on the excess of dextrose itself, or indirectly on the appearance of decomposition products (e.g., lactic acid) produced from it, will come into play, and almost certainly throw out of working order those mechanisms which under ordinary circumstances would take care of any physiological excess of dextrose in the blood.

The main difference between our own and Kleiner's results is that we have found the liver to be capable of removing some of the excess of dextrose, whereas Kleiner did not. It might be raised as an objection to our results that the lower percentage of dextrose in the blood of the vena cava over that in the portal vein was merely due to the dilution which the blood, flowing into the cava from the liver, suffered on mixing with the blood meanwhile ascending from the leg veins and to its dilution by the blood of the hepatic artery. Although we are prepared to admit that such dilution may account for part of the decrease, we feel confident that it is an unimportant factor because: (1) the veins of one kidney and one hind leg were ligated, and (2) in one of the three experiments of this group there was a higher percentage of sugar in the muscle-blood than in that of the vena cava opposite the liver.

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As has been indicated above, one object of the above observations was to secure suitable experimental conditions upon which to investigate the influence of various factors on the assimilation of dextrose. Of these we have so far investigated the influence of alterations in the reaction of the blood.

Before proceeding to indicate the general nature of the results, it may be well to point out that there has been an accumulating mass of evidence during recent years tending to show that the behaviour of carbohydrate in the animal body is decidedly influenced by the reaction of the tissue fluids. Thus, in dyspnea, where the H-ion concentration of the blood becomes increased, hyper-

glycemia and glycosuria are commonly observed, and after the administration of acids by stomach the assimilation limit of dextrose becomes much depressed. Hyperglycemia is also more readily established when acid and dextrose are ingested than when a corresponding quantity of dextrose is given alone, and glycogen accumulates in the liver to a much less degree (Elias<sup>9</sup>). On the other hand, when alkalis are administered intravenously to normal rabbits, a decrease frequently occurs in the percentage of blood-sugar (Underhill<sup>10</sup>), and when given to dogs rendered diabetic by extirpation of the pancreas, the glycosuria almost, if not entirely, disappears (Murlin and Kramer<sup>4</sup>). Furthermore, adrenin does not cause so marked a degree of hyperglycemia when it is administered to rabbits following the injection of sodium carbonate as it does in normal animals (Underhill).

In view of these results the question arises: upon what sugar-disposing function of the body does the acid or alkali exert an influence? Two possibilities are to be thought of: it acts either (1) on the storage (polymerization) of sugar as glycogen in the liver and muscles, or (2) on the oxidation of sugar in the tissues. To throw light on this problem Murlin and Kramer examined the respiratory quotient,  $\text{CO}_2$ , of normal and diabetic dogs under the admin-

$\text{O}_2$

istration of sodium carbonate and dextrose. An increase in the quotient (towards 1.0) indicates increased combustion of dextrose, and although such was not found to occur when alkali was given to a normal animal, it did occur when alkali was given intravenously along with dextrose to one that was diabetic. The conclusion drawn from these results is that the alkali stimulates the oxidation of dextrose in the tissues, a process which, however, might be taking place at the same time as one of a greater storage of other molecules of dextrose as glycogen. Kramer and Marker<sup>11</sup> have however recently contributed evidence which would seem to indicate that such increased glycogen formation does not occur under the above conditions. These authors found that glycogen did not appear in the liver following the administration of the alkali, nor by giving adrenin to a depancreated dog that had been retaining dextrose while on a meat diet, were they able to cause an increased excretion of dextrose in the urine.

These very significant observations concerning the beneficial influence of alkali administration on that form of experimental diabetes which most closely simulates the more acute varieties of the disease in man prompted us to investigate, by the methods above described, whether or not sugar-storage by liver and muscles could be stimulated by injections of alkali (sufficient to perceptibly lower the H-ion concentration of the blood), and whether acids would have the opposite influence.

Details of our results will be published elsewhere, and we will accordingly only briefly offer a preliminary notice of some of them here. In the first place, it was noted that when neutral dextrose solutions are injected intravenously, there is a distinct tendency for the H-ion concentration of the blood—as measured by the colorimetric method—to become increased. This is possibly due to the production of decomposition products of the dextrose (lactic acid); at least we have been able to show that the blood of such injected animals contains about twice as high a percentage of lactic acid as that of normal animals. Even



when, along with the dextrose, moderate quantities of sodium carbonate are also injected, there may be distinct (colorimetric) evidence of increasing blood acidity, so that to really produce an alkalosis, when dextrose is being injected, very large injections of alkali are necessary. When enough alkali was injected to distinctly depress the H-ion concentration of the blood, it was usually found that the liver more actively removed sugar from the blood flowing through it than ordinarily. By injecting a sufficient amount of alkali into dogs whose blood already contained an excessive concentration of dextrose, as a result of previous feeding with excess of carbohydrate and operative manipulation, a distinct lowering of the blood-sugar concentration became evident, but it could not be shown, by comparison of the curves representing this in the portal vein, vena cava and iliac veins, whether the liver or muscles were mainly responsible for the disappearance.

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## PITUITARY STANDARDIZATION\*

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THE value of pituitary extract (posterior lobe) as an effective therapeutic agent for controlling blood pressure and for stimulating uterine contractions in the second stage of labor is universally recognized. Because of these important uses it is necessary that the extract be standardized as accurately as possible.

The crude materials from which valuable substances are obtained are unfortunately very variable. This, however, is of slight importance compared to the effect that the various chemical and pharmaceutical manipulations, incident to the preparation of these substances, have on their activity. The adoption of a high standard of activity and rigid adherence to the same is imperative for a product of such great physiologic importance as that of pituitary extract. In the case of this extract, as is also true of extracts of some other glands and crude drugs, the chemical constituent to which it owes its therapeutic value has not been isolated.

In such cases advantage is taken of the fact that active medicinal substances when administered to animals have characteristic physiologic effects which are more or less typical and which can be used as assay reactions. This procedure is similar to the use of chemical reactions on which to base chemical assay processes. Biologic standardization is now generally recognized as an indispensable adjunct to the commercial valuation and to the scientific investigation of certain substances of great importance in medicine.

When several physiologic effects are more or less characteristic of the substance in question, differences of opinion naturally prevail as to which is best adapted to an accurate quantitative assay process.

It is the intention of the authors to describe the two methods that have been proposed and used for the assay of the various substances and extracts derived from the pituitary gland, and to point out the advantages and disadvantages of each as these have developed under practical working conditions.

The two methods referred to above are those known as the blood pressure and the oxytocic tests.

In a former communication<sup>1</sup> some of the physiologic effects following the administration of extracts of the pituitary body were reviewed and discussed as to their adaptability to the purpose of standardization.

Of the various characteristic effects, that on the circulatory system was considered most constant and subject to the least variation due to causes other than differences in the amount administered. This effect was, therefore, adopted as an assay reaction to determine the activity of solutions prepared directly or indirectly from the infundibulum of the pituitary gland.

### THE BLOOD PRESSURE METHOD.

The method of assay in brief is as follows: A dog weighing approximately 10 kilos is anesthetized, preferably with chlorctone, and is prepared for test pur-

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poses by inserting a cannula into a femoral or other convenient vein for injecting the solution, and another cannula into one of the carotid arteries. By means of a flexible connection the artery is joined to a manometer attached to a kymograph, to obtain a record of the normal heart action and blood pressure for comparison with that following an injection of the active extract. The standard originally<sup>1</sup> used for comparison in making such assays was the activity obtainable from the dried, defatted, powdered, infundibular portion of the bovine glands. Gland material so prepared seems to retain its activity indefinitely and to permit of quick and complete extraction by simple infusion. Later, however, the possible objections to this have been obviated by the use of a stable and highly active water-soluble powder prepared by Aldrich.<sup>2</sup> This was carefully assayed and its activity compared with that of the former standard. The Standard Test Solution is prepared from this, using a sufficient quantity of the powder to make a solution containing in 1 c.c. the highest activity found in 1 mg. of the dried gland material referred to above.

The technic of the test is to inject the standard test dose—1 c.c. of the Standard Test Solution—into the vein, note the rise in blood pressure, and after 15 minutes repeat the injection. It is some times necessary to repeat this injection two or three times before one obtains an equal change in blood pressure from two consecutive injections of the test dose.

The standard and sample are then injected alternately with intervals not less than 15 minutes, varying the amount of the sample, if necessary, until the rise in blood pressure is the same from definite quantities of each. The activity of the sample can be deduced from the amounts used and is stated in terms of the Standard.

The following points must be carefully observed as a means of making this an accurate quantitative method for the valuation of pituitary extracts:

1st. The dog must be healthy and should maintain a good normal blood pressure.

2nd. It must be completely anesthetized until all reflexes are destroyed.

3rd. It must be sensitive to changes of 10 per cent in the amount of pituitary extract injected. For example, if doses of 0.9, 1 and 1.1 c.c. of the test solution induce the same amount of change in blood pressure with no measurable differences between them, that dog is not in a condition satisfactory for standardization work.

4th. It must be sufficiently sensitive to pituitary extract so that 1 or at most 2 c.c. of the Test Solution (q.v.) raises the pressure at least 1 cm.

5th. Two consecutive injections should have at least 15 minutes interval between them.

The method just described was adopted tentatively until a more logical or accurate one could be developed.

#### THE ISOLATED UTERUS TEST.

The increasing use of pituitary extract in obstetric work, suggested a much more logical method of assay than the one described, namely, a test based on the action of pituitary extracts on the uterus muscle. Such a method was used and first described by Dale and Laidlaw<sup>3</sup> and afterwards by various investigators.

notably Fühner<sup>1</sup> and Guggenheim.<sup>5</sup> The experimental work has been exhaustively summarized by Roth<sup>6</sup> who assayed eleven commercial samples and reported his results.

The great differences in activities of the samples examined led him to the conclusion that "the statement 'physiologically standardized' means practically nothing." Roth, however, apparently overlooked the fact brought out by his own tests that in every case the two samples from the same manufacturer had the same activity, indicating that each laboratory although having a different standard had adhered to it.

The fault, if any existed, was not in the lack of a standard nor in a failure to apply standardization tests, but in the failure of the manufacturers to co-operate in choosing a single standard.

Roth, in a previous article<sup>7</sup> proposed the use of  $\beta$ -iminazolyethylamine, commonly called histamine, as a standard for measuring the activity of pituitary extracts. His proposal now reappears as the official standard for a U. S. P. solution of hypophysis extract and is compulsory to the extent that extracts standardized in any other way do not meet official requirements. While a very dilute solution is required for standardization purposes, the present almost prohibitive price of histamine, due to the fact that the supply in this country is very limited, tends to preclude its use as a standard.

Such a standard is also open to question from two viewpoints. Are different lots of histamine equal in their action on the uterus? Is a substance which has no practicable value in obstetrics,<sup>8</sup> when used alone, a true standard for determining the activity of a powerful oxytocic agent such as pituitary extract, and is it a proper standard when the action in question is as a pressor agent? If the value of pituitary extract were only as an oxytocic agent or if only that portion applied to obstetrical purposes were to come under the U. S. P. ruling then some of the objections to the method and the standard would be removed. Under present conditions, however, a more commendable standard would be a substance prepared from the gland and having the same qualitative action as fresh glandular extracts.

While the details of the technic and equipment for this test vary in different laboratories, the essentials are briefly as follows: One horn of the excised uterus of a young virgin guinea pig is used, it being suspended between a fixed point, and the end of a movable lever. It is bathed in Locke's solution which is kept at a uniform temperature of 38° C and constantly aerated with air or oxygen.

The solutions to be tested are mixed with the Locke's solution in which the muscle is suspended and only the small part in contact with the strip of uterus is responsible for the effect produced. The exquisite sensitiveness of this muscle to the action of stimuli such as an active pituitary extract, is shown by the fact that some specimens are sensitive and respond to the stimulus of the infinitesimal amount which comes in contact with them from a solution containing only 1 part in 100,000,000. This shows too the exceedingly powerful character of the active principle of the gland, for although the substance referred to above was highly purified, it did not possess the physical characteristics of, and probably was not a pure principle.

Without entering into a detailed description of the method by the authors,

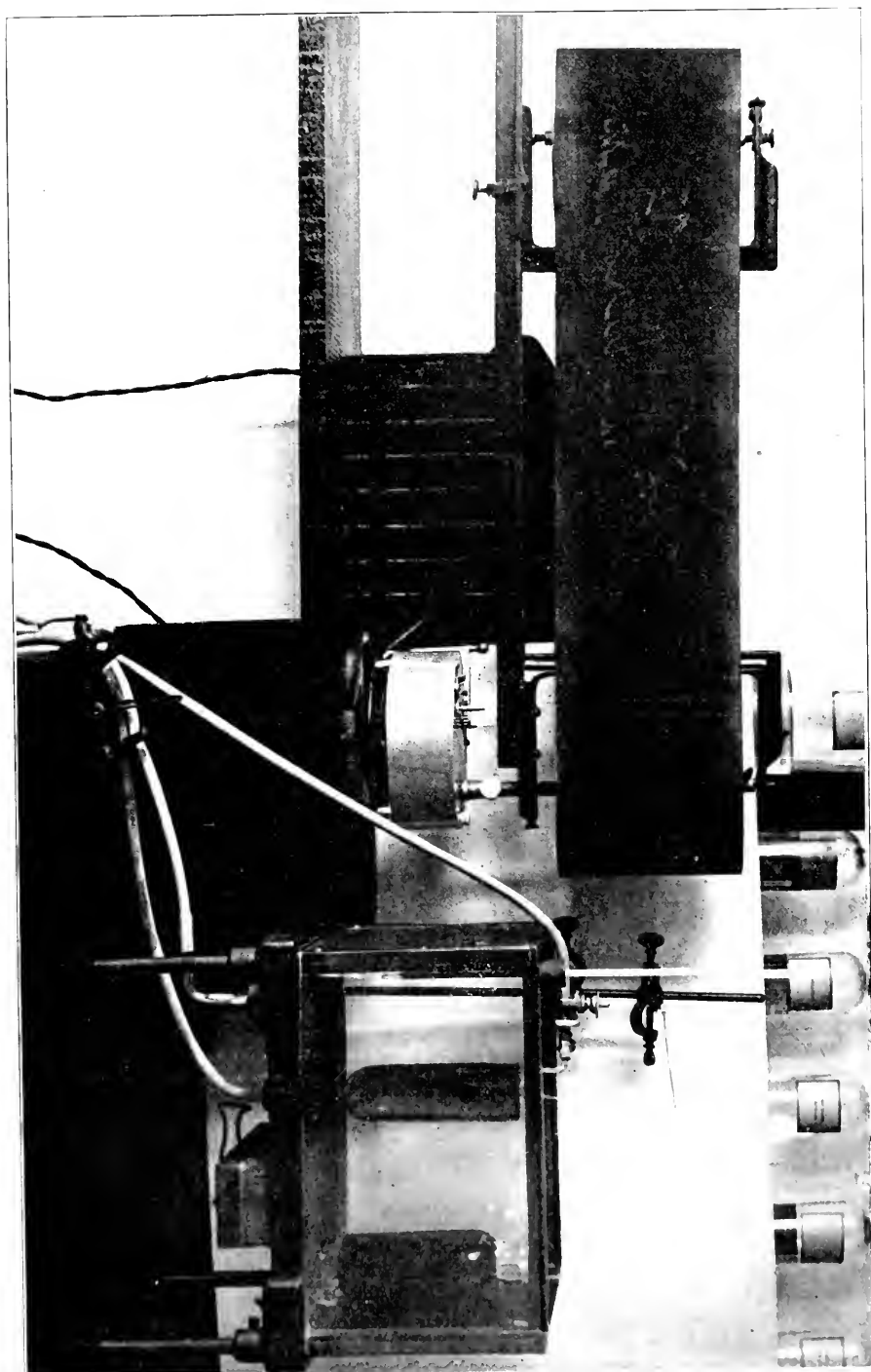


Fig. 1.—Complete equipment for oxytocic test.

which is outlined above, some points will be especially considered because so much depends on the carefulness with which they are followed.

*The Test Animals.*—While differences of opinion exist as to the one best adapted, most investigators have agreed on the young virgin guinea pig weighing 250 to 350 gms. and rejecting those in the oestral stage.

*The Organ.*—One horn of the uterus using a strip 1 to 2 cm. long. It should be normally slender and not congested. Congested, thickened uteri, while often very sensitive, are too irritable for standardization tests.

*The Apparatus.*—This includes the kymograph, a writing lever, a constant temperature bath accurately adjusted to hold a temperature of  $38^{\circ}\text{C}$ , an inner container for Locke's solution in which the uterus muscle is suspended, and facilities for preheating the Locke's solution to the same temperature as that of the bath, and for its aeration (see Fig. 1).

The heating of the constant temperature bath can be done accurately and conveniently by the method described by Dale and Laidlaw, a gas flame striking a copper rod which passes into and around the outer vessel. The apparatus in use at present and which has proved very satisfactory for maintaining a uniform temperature consists of a rectangular vessel of 8 liters capacity which is heated from below by an electric heating coil enclosed in a soapstone box. The amount of current is regulated by a rheostat and when once adjusted will maintain the temperature of the water bath at  $38^{\circ}\text{C}$  provided the room temperature remains fairly constant. The large outer vessel has a soapstone bottom covered inside with copper while two sides are made of double walled glass with an air space between. The apparatus is well adapted to the purpose intended and is very simple in construction and operation.

The preheating of Locke's solution is most conveniently done by keeping a supply in another container in the outer bath in which it attains and holds the same temperature as that in contact with the muscle. (Note bottle to right in large vessel.)

The aeration of the muscle is best secured by the method suggested by Dale and Laidlaw, conveying a current of air or oxygen through the tube (leading to top of inner container) which forms the lower support of the muscle, the upper end of the muscle being attached by thread or otherwise to the writing lever.

The writing lever should be light, such as a straw or an aluminum wire, should move almost without friction, and magnify the contractions 3 to 5 times.

Not of least importance is the Locke's solution which should be made of the best quality "reagent" chemicals.

*The Technic.*—This includes weighting the muscle to counteract the excessive contractions of an irritable uterus; injecting into the Locke's solution quantities of sample and standard which will induce equal contraction of the uterus less than the maximum for the specimen; maintaining in the meantime a constant temperature, removing spent pituitary solution; washing the specimen, and repeating injections not too often to destroy its tonicity. Injections can best be made by means of a hypodermic syringe which forces the dose into the solution and tends to mix and make the solution homogeneous almost immediately. This is aided by the continuous bubbling of air through the solution. When the

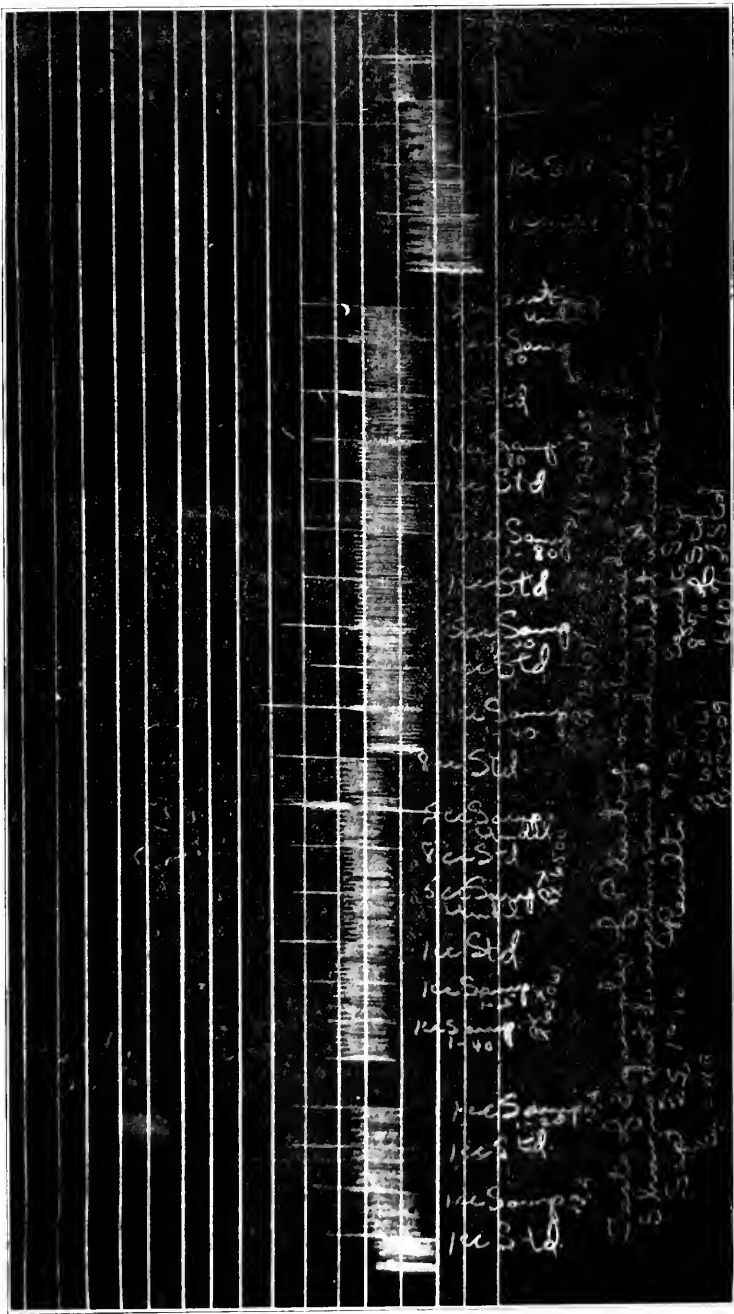


Fig. 2. Twenty-four consecutive injections of pituitary extract into the same dog.

maximum contraction for any dose has been reached and no further rise of the writing lever takes place, the spent solution is withdrawn through a valve at the bottom of the inner container passing through the bottom of the outer bath. The specimen is washed with fresh Locke's and an additional quantity is left in contact filling the container to some determined volume. Another injection can be made as soon as the muscle has relaxed to normal length.

#### DISCUSSION OF METHODS.

The oxytocic test on the uterus muscle is without question more logical than the blood pressure effect for standardizing substances used almost exclusively as oxytocic agents, unless there is evidence that the same active constituent is responsible for both effects. If this is true in any particular instance, then accuracy and convenience only need be consulted. Opinions differ regarding this point as applied to the pituitary gland but the most generally accepted one is that there are several active bodies with different physiologic actions. In other words an extract of the gland might raise the blood pressure without having any oxytocic activity. This is a point which requires more than passing consideration. Because of the fact that a good specimen of the isolated uterus of a guinea pig is sensitive to a much smaller quantity of pituitary extract than can be detected by the blood pressure test and that such a test organism is also very sensitive to other substances and to variations in the test conditions, it is very difficult to prove that there is both a pressor principle and an oxytocic principle in the glands. The authors have not yet found any data to support the contention that the two actions are not due to an identical substance. Any chemical treatment to isolate one from another either destroys both actions or affects them equally.

Another objection urged against the blood pressure method as of noteworthy importance,<sup>6</sup> is the statement that repeated injections of pituitary extracts, even when prepared from the separated infundibulum, induce progressively decreasing pressor effects until the depressor predominates and no rise in blood pressure follows the injection of the active extract. This, however, is not a valid objection when the test conditions outlined above are followed carefully.

In many cases between 20 and 30 doses of 0.05 c.c. Pituitrin or an equivalent quantity of similar preparations have been injected intravenously into dogs without the depressant effect becoming noticeably increased. (See tracing, Fig. 2.) If large doses were used and the injections followed each other closely the objection noted would be a valid one. All physiological testing, however, must be done under rigidly prescribed conditions, otherwise there is little accuracy.

In this method of testing, the test animal is the dog admitted by all to be the least subject to the so-called tolerance; the doses are small; they follow each other at intervals not less than 15 minutes; sample and standard are injected alternately and must give an equal rise in pressure, the rise in pressure from the standard test dose must be 10 mm. or more. Under such conditions the method is satisfactory.

McCord<sup>9</sup> demonstrated that the isolated organs except the heart respond almost without fatigue to repeated injections of pituitary extracts, the effect in nearly every case being markedly constricting on the muscle walls. The heart was depressed and in a very large majority of the experiments there resulted a



decreased rate and amplitude. These results seem to indicate that the pressor effect on the vessels is fairly constant, and discredit any theory of a pressor principle distinct from a depressor principle.

This is corroborated by Roth<sup>6</sup> in the statement: "By the blood-pressure method, a comparison could easily be made between Sample 1 (Pituitrin, P.D. & Co.) and Sample 3 (Infundin) since the pressor response was little diminished even after hours when weak dilutions were used."

Roth further states, however, "On the other hand, it was very difficult to compare Samples 6 (Hypophysin) with Sample 1 (Pituitrin, P.D. & Co.), and impossible to compare Sample 8 (Pituitary Ext. Schering) with Sample 1." This statement has not been verified because if each is injected in sufficient quantity to give equal heights of blood pressure, the activities are certainly in inverse ratio to the dilutions used. If a preparation fails to raise the blood pressure, but with a dose  $7\frac{1}{2}$  times as large as another sample, has an equal effect on the isolated uterus, who can say that the latter and not the former is the true index to its therapeutic value?" If an extract is so poorly prepared that a large quantity is needed to raise the blood pressure measurably, deleterious substances may be present in such a proportion as to obscure the pressor effect. However, would not these other substances have an equally obscuring effect on the oxytocic test?

The foregoing statements regarding the objections raised against the blood-pressure method are not intended to be taken as refuting them but to explain why the authors do not consider them so vitally important and to lay the facts open for general consideration. The fact that the oxytocic test and the comparison with histamine as a standard are now incorporated in the 9th Revision of the United States Pharmacopeia would indicate that these objections have been either answered or overlooked, possibly the latter since those most interested in the subject were ignorant of this contemplated step until it was accomplished.

The isolated uterus test, while in some respects logical, is not in its present form practicable to be used as a commercial method for the final standardizing of pituitary extracts. It is a good qualitative test but lacks the accuracy and dependability necessary for commercial quantitative standardization.

While apparently simple both in technic and in the apparatus needed, experience has proved that in the former, at least, it is quite the contrary; the large number of pigs that are not adapted to the test, the slow and unsatisfactory preliminary standardization of the uterus, and the inaccuracy of the test when applied to weak extracts are objectionable features as great as, if not greater than, the objections advanced against the blood-pressure method.

In attempting to apply this method there were weeks at a time when some unfavorable conditions prevented our obtaining results allowing any degree of comparison. Compare also Dale and Laidlaw (*loc. cit.*).

In some cases the uterus is so sensitive as to respond beyond the limits of measurement to minute quantities of an extract. Weighting the lever to overcome part of this sensitiveness often results in killing the sensitiveness even when this is done with the greatest care.

On some occasions (and this is the most frequent cause for rejecting a

specimen) it is impossible to obtain two equal consecutive contractions of the uterus from equal amounts of the same extract.

At times the return to normal after a contraction is so slow that whether one waits for spontaneous relaxation or attempts to counterbalance the contraction with weights or bring about relaxation by means of an adrenalin solution, the sensitiveness of the specimen is lost.

That the difficulties of the test have been discovered by every investigator is evidenced by the fact that there is little agreement among them in the description of the technic employed. Guggenheim claims that the rat is free from some of the objections charged to the guinea pig. Roth claims that the uterus of a virgin guinea pig is better than that from dog, cat or mouse. He also claims that for only about two hours can a uterus specimen be used for this work while Fühner could use the same strip for a week.

The latest report on this point is that of Fenger, which appeared since writing this article,<sup>10</sup> who states that "The selection of a suitable uterus is not always an easy matter. Often three or four pigs have to be killed before a satisfactory strip is obtained. The individual sensitiveness towards pituitary and histamine varies considerably. It is, consequently, by comparing several tracings on different days that a fairly correct conclusion in regard to actual strength of a certain solution can be drawn. It would be advisable to obtain a standard which resembles more closely the active principle of the posterior lobe than does histamine."

With the strictest attention to details some of the objections have been minimized but when the test can not be made more accurate than merely to distinguish between dilutions, 1-1000, 1-2000 and 1-3000 [the middle one being considered equal to standard and the others credited with respectively greater and less contractions than standard (see reference 6)], its accuracy leaves much to be desired.

While apparently the oxytocic test on the uterus muscle is the more logical one for standardizing pituitary extracts intended for obstetrical use, one cannot but question whether it more certainly indicates oxytocic activity than does the blood pressure test. Several substances such as blood serum, beef bouillon, peptone, egg white, putrid meat extracts, act on the uterus muscle without having any recognized oxytocic value.<sup>11, 12</sup> In the case of pituitary extract, is it more than merely a coincidence that it acts on the excised uterus muscle and also on the gravid uterus? Agents with oxytocic value are, without exception, those which react on smooth muscular tissue; the arterioles and small veins, as well as the uterus, belong to this class of muscle. It is the action of pituitary extracts on these unstriated muscles which brings about increased blood pressure. This seems, therefore, to be a logical test reaction. In this connection another point should be given due consideration—the increasing use of pituitary extract for its blood pressure effect particularly during or after surgical operations. By its use the systemic pressure is increased while the pressure in the pulmonary circuit is lowered—a condition described by Wiggers<sup>13</sup> as "a fortunate combination of actions." Shall we apply the oxytocic test exclusively to a substance so valuable for its pressor effects?

From Roth's results<sup>6</sup> we tabulate the following comparisons of pressor and uterine values:

SAMPLE.	VALUES.	
	PRESSOR.	UTERINE.
1	15	7.5
6	1	1
8	?	1

On the face of this report it would seem possible for the obstetrician to determine whether Sample 1 is 15 times or only  $7\frac{1}{2}$  times as active as Sample 6. More striking still is the discrepancy in the case of Sample 8 of which Roth injected 250 times as much as of Sample 1 without an equal rise in blood pressure while the uterus test would indicate that it was not devoid of activity. This again is contrary to the statements of Dale and Laidlaw<sup>3</sup> that the pressor and oxytocic effects seem to go hand in hand, an observation made also by Frankl-Hochwart and Fröhlich<sup>14</sup> and repeatedly confirmed in our own work.

The problem seems to be one not for the pharmacologist alone, but for the clinician as well.

While we are not condemning as useless the uterus test for standardizing pituitary extracts, we are not willing to subscribe to the popular opinion that this test alone is deserving of recognition, that it has greater accuracy as a standard method of assay than the pressor test, or that the latter is not a measure of oxytocic activity.

We conclude, therefore:

First, that neither method in its present form is ideal as a means of standardizing pituitary extracts.

Second, that most specimens of the isolated guinea pig uterus are sensitive to the action of these extracts, some are exquisitely sensitive but few are sufficiently uniform in the reaction to be used for accurate standardization purposes.

Third, that the pressor test is a fairly accurate measure of pituitary activity, is not an illogical indicator of oxytocic value, and is free from some of the most objectionable features of the uterine method.

We regret our inability at the present time to add anything to the refinement of either method but suggest first, that the possibilities of the pressor test be further developed since it has been found capable in our hands of recognizing differences in pressor activity of less than 10 per cent; second, that chemists, pharmacologists, and clinicians work together in an effort to determine whether pressor and oxytocic values depend on the same active principle.

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# LABORATORY METHODS

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## REVIEW OF THE FOLIN AND DENIS METHODS FOR THE DETERMINATION OF NITROGEN BY DIRECT NESSLERIZATION

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THE colorimetric methods for the determination of nitrogen in the blood and the urine have required that the ammonia produced by the destructive digestion with sulphuric acid or by catalyzers, such as urease, be removed from the digestive mixtures by aeration. This procedure besides requiring some time is difficult to carry through with certainty that all the ammonia is recovered, so that the publication of a method by Folin and Denis\* for the direct Nesslerization of the ammonia in such mixtures is very welcome and of great value. Folin and Denis have described methods for the estimation of—(1) the total nitrogen in the urine, (2) urea nitrogen in the blood and urine, (3) urinary ammonia, and (4) the nonprotein nitrogen in the blood. The methods are briefly given here. The directions follow closely the concise descriptions given by Folin and Denis, and it is urged that they be followed accurately, since any alteration may lead to error, as the authors point out in the original paper.

### TOTAL NITROGEN IN THE URINE.

The presence of large amounts of sulphates in the digestion mixture, following the destructive digestion of the urine with sulphuric acid was the chief obstacle in the direct Nesslerization of the ammonia contained therein. Folin and Denis have discovered that the presence of phosphoric acid in the digestion mixture not only hastens the oxidation but also allows direct Nesslerization of the ammonia.

### SOLUTIONS AND APPARATUS REQUIRED.

*Nessler Reagent.*—This is prepared as follows: Seventy-five grams of potassium iodide are dissolved in 50 c.c. of warm distilled water and 100 grams of mercuric iodide added. The mixture is stirred until the solution is complete. The solution may be a little turbid. It is then diluted with four or five hundred cubic centimeters of water, filtered, and the filtrate made up to one liter. This is the stock solution from which the dilute Nessler reagent used in direct Nesslerization is made by adding to 300 c.c. of the above solution 200 c.c. of a 10 per cent sodium hydrate solution and 500 cc. of water. Fifteen cc. of this dilute Nessler's solution added rapidly to solutions containing the amount of ammonia in the various colorimetric determination will yield crystal-free mixtures.

*Ammonium Sulphate Solution.*—Folin and Denis recommend Kahlbaum's C. P. ammonium sulphate for the preparation of the standard. This is pure but

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\*Folin, Otto and Denis, W.: Nitrogen Determination by Direct Nesslerization, Jour. Biol. Chem., September, 1916, xxvi, 473 to 506.

must be dried in a desiccator for several days, or by heating it to about  $110^{\circ}$  C. for an hour before it is weighed out. The standard is made as follows: 4.716 grams of the salt is weighed out accurately and dissolved in one liter of 0.2 normal sulphuric acid. The solution contains one milligram of nitrogen per c.c. and keeps very well. From this solution appropriate dilutions can be made.

*Sodium Hydrate Solution.*—100 grams of sodium hydrate are dissolved in one liter of water.

*The Digestion Mixture.*—300 c.c. of ordinary phosphoric acid "syrup" (containing about 83%  $H_3PO_4$ ) are added to 100 c.c. of concentrated sulphuric acid. To this mixture 15 c.c. of a 10% solution of copper sulphate are added and the solution is filtered.

*Pipettes and Glass Ware.*—A long stem calibrated Oswald pipette, which delivers accurately 1 c.c. of water when drained for ten seconds against the side of a test tube and then blown clean, is required for measuring the urine. An ordinary 1 c.c. pipette, 200 or 250 c.c. volumetric flasks, micro-burners, hard glass test tubes (200 mm.  $\times$  13 mm.) and a Duboscq colorimeter, the zero point of which has been determined with care, complete the necessary equipment.

*The Determination.*—The urine is diluted so that 1 c.c. contains from 0.7 to 1.5 mg. nitrogen. In general, the following rules may be applied: Dilute urines having specific gravities of less than 1.018, five times; urines having specific gravities between 1.018 and 1.030, dilute ten times, and those whose specific gravities are above 1.030, dilute twenty times.

One c.c. of the diluted urine is measured by the Oswald pipette into a large test tube, and 1 c.c. of the phosphoric-sulphuric acid mixture is added. A fresh bit of granite is introduced to prevent bumping. The tube is then heated over a micro-burner, until nearly all the water has been driven off as indicated by the absence of foaming or by the appearance of denser sulphuric acid fumes within the test tube. The mouth of the test tube is then covered, and the heating continued with the flame regulated so that only a little of the acid fumes escape from the tube. In one-half to three minutes, counting from the time the test tube is closed, the digestion should be clear and blue, green, or light yellow in color. This color is due to the copper. The heating is continued for 30 to 60 seconds, but in no case should the time of heating be less than two minutes after the closure of the tube.

The test tube is then allowed to cool for two or three minutes and a little water is added and the contents rinsed into a 200 or 250 c.c. volumetric flask, using about 150 c.c. of water for the purpose.

The titratable acid content of 1 c.c. of the phosphoric-sulphuric acid mixture is now determined using 10% sodium hydrate with phenolphthalein as an indicator. The number of c.c. of alkali used in neutralizing the acid is then multiplied by 1.375. This figure represents the number of c.c. of alkali which must be added to the digestion mixture to make it neutral. Two c.c. in excess of this amount are added to make the solution definitely alkaline.

The standard solution is prepared as follows: Into another volumetric flask of the same capacity as that used in the case of the unknown, introduce 1 c.c. of the sulphuric-phosphoric mixture and 20 c.c. of an ammonium sulphate which is prepared from the standard stock solution by diluting it twenty times.

(The diluted solution contains 0.05 mg. nitrogen per c.c., and is a dilution quite appropriate as a standard for most nitrogen determination in blood and urine.) About 125 c.c. of water and the same amount of alkali that was used in the case of the unknown is then added and thoroughly mixed. Then to each flask 15 c.c. of the dilute Nessler reagent is added from a cylinder and the contents thoroughly mixed. The flasks are filled to volume and again mixed. A small part of the unknown is then poured out as an added precaution against incomplete mixing, and a portion of the remainder is filtered through a cotton plug or centrifuged. If the sediment thus obtained is mixed with a red deposit the Nesslerization is faulty and the fluid must be discarded. The liquid above the sediment must be clear and not "smoky." This fluid is to be used in comparison with the standard as follows:

Both cups of the colorimeter are filled with a portion of the standard and the colorimeter set at 20 mm. Adjust the cups to the same color intensity. When this is done, replace the fluid in one of the cups with the unknown and make one leisurely careful reading. This method of reading the colorimeter has been found to give the best results. As stated above the color of the standard is given by 0.05 mg. of nitrogen per 100 c.c. The calculation of the unknown is

$$\frac{20}{\text{reading of the unknown}} \times .05 = \text{mg. nitrogen in 1 c.c. of urine.}$$

#### THE DETERMINATION OF THE NONPROTEIN NITROGEN OF THE BLOOD.

Folin and Denis discovered that by using *m*-phosphoric acid as a precipitant for the blood proteins, the nonprotein nitrogen of the blood can be determined by direct Nesslerization in a manner similar to that just described. The method is as follows:

Since *m*-phosphoric acid (glacial phosphoric acid) is not stable in watery solutions; it should be prepared fresh from the sticks. The sticks weigh about 25 grams and will dissolve in 100 c.c. of cool water in an hour's time. The solution thus obtained can be used for two or three days. The other solutions required are the same as those used in the estimation of the total nitrogen in the urine.

Into 50 c.c. volumetric flask containing 20 c.c. of water, 5 c.c. of blood along with 3 c.c. of the 25% *m*-phosphoric acid solution are introduced and mixed thoroughly. After allowing the mixture to stand for an hour the flask is filled to the mark, and the contents filtered through dry filter paper. If the first few drops are not water-clear, they must be discarded or put back upon the filter. If time is a factor it is not necessary to allow the mixture to stand an hour. In this case the volume is made up at once and the contents transferred to a larger flask, which is vigorously shaken for three to five minutes and filtered. Clear filtrates can be obtained in this way.

Ten c.c. of the clear filtrate, corresponding to 1 c.c. of blood, are placed in a large test tube and after the addition of 1 c.c. of the phosphoric-sulphuric acid mixture, the tube is heated over a micro-burner exactly as described for the urinary nitrogen. When the digestion is completed the contents of the test tube are rinsed into a 100 c.c. volumetric flask, using about 60 c.c. of water. The titrating value of the phosphoric-sulphuric acid mixture is obtained as

described above, and 1.375 times the amount plus 1 c.c. for alkalinity of 10% sodium hydrate is added. The flask is cooled and 10 c.c. of the dilute Nessler reagent is added, and the flask filled to the mark. Filter or centrifuge, if necessary, and the nitrogen is determined with the colorimeter, using as a standard the ammonium sulphate solution as described in the total urinary nitrogen method.

In cases where there is a very high nonprotein content, a standard of 1 mg. ammonia nitrogen may be required. In working with such blood the standard is best prepared as follows: The acid is diluted and the alkali is added to the flask which is to contain the standard, without adding the standard ammonia, until the color has been developed in the unknown. By inspection it can readily be told after a little practice how strong the standard should be, and the standard ammonia added accordingly.

#### THE DETERMINATION OF AMMONIA IN THE URINE BY DIRECT NESSLERIZATION.

The presence of creatinin in the amount that it is found in normal urine prevents the direct determination by Nesslerization of its ammonia. Folin and Denis have determined that if the amount of creatinin be reduced to a trace or even a large trace, direct Nesslerization is possible. This they do by the use of blood charcoal. The determination is carried through as follows:

Ten c.c. of urine, along with 1 c.c. of *m*.-phosphoric acid (25%), 9 c.c. of water, and 2 grams of blood charcoal (Merck's), are placed in a large test tube and vigorously shaken for a minute or so. The contents of the tube are filtered through dry filter paper, and 1 to 5 c.c. of the filtrate transferred to a 100 c.c. volumetric flask. About 70 c.c. of distilled water is then added and 15 c.c. of the dilute Nessler reagent previously described is quickly introduced. The contents of the flask is thoroughly mixed and made up to 100 c.c. A portion of the clear solution obtained is then compared in the colorimeter with the standard ammonia solution which has been treated with dilute Nessler reagent as previously described in the estimation of total nitrogen in the urine.

#### THE DETERMINATION OF THE UREA IN THE BLOOD AND THE URINE BY DIRECT NESSLERIZATION.

By using a 25% solution of *m*.-phosphoric acid as a precipitant of the urease materials used in the hydrolysis of urea, it is possible to determine the urea in the blood and urine by direct Nesslerization, as in the case of the methods previously described.

The urease can be prepared readily by rubbing up 5 grams of soy bean meal (The Soja bean meal sold as a food for diabetics can be used) with 5 c.c. of water; 400 c.c. of water and 100 c.c. of alcohol are then added. 10 to 15 c.c. of the suspension obtained are used in each determination. The preparation remains good for two days at room temperature.

The urea in the blood is determined as follows: 5 c.c. of blood and 10 c.c. of the suspension of soy bean meal are placed in a 50 c.c. volumetric flask, which is stoppered and allowed to stand for an hour. 25 c.c. of water along with 2 c.c. of the *m*.-phosphoric acid (25%) are then added and the flask made up to volume. The contents are thoroughly mixed and allowed to stand for 45 minutes or over night, after which they are filtered. A half gram of blood

charcoal is added to the filtrate, which is thoroughly mixed and again filtered. In the case of normal or approximately normal blood, transfer 10 c.c. of the last filtrate (corresponding to 1 c.c. of blood) to a 25 c.c. volumetric flask, and add 5 c.c. of the Nessler's reagent, make up to volume, mix, and compare at once in a colorimeter with a 0.25 mg. ammonia nitrogen solution (standard ammonia solution diluted one to four), Nesslerized in a 50 c.c. flask with 10 c.c. of Nessler's reagent. If less blood is used, a larger portion of the filtrate can be used for Nesslerization. The determination of the urea in the urine is not essentially different than that used in the determination of the urea in the blood.

One c.c. of urine is transferred with an Oswald Pipette to a 100 c.c. volumetric flask, along with 15 c.c. of the soy bean suspension. After allowing the mixture to stand for an hour, 25 c.c. of water and 1 c.c. of 25% *m.*-phosphoric acid are added and thoroughly mixed. One gram of Merck's blood charcoal is then introduced and about 25 c.c. of water. The contents are then thoroughly mixed, and made up to volume and filtered. From 5 to 20 c.c. of the clear filtrate are transferred to a 100 c.c. measuring flask (the amount should contain from 0.7 to 0.13 mg. nitrogen). Dilute with water to about 60 c.c. and Nesslerize in the usual way, and compare with a standard in the colorimeter. The standard should contain, in the majority of cases, 1 mg. nitrogen.

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## URINALYSIS IN RELATION TO RENAL LESIONS\*

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BY J. R. STARK, M.D., CINCINNATI, OHIO.

THE object of the work upon which this paper is based, was to discover what relation exists between urinalysis, the clinical diagnosis of nephritis, which is so frequently directly based upon urinary findings, and the anatomic changes in the kidneys. The material which served as the basis consisted of six hundred clinical histories in connection with which the term nephritis was used in the clinical diagnosis. All of the cases studied had gone to autopsy and therefore the anatomic renal material was complete. The clinical history, autopsy protocols, gross material, and microscopical preparations were therefore available for study.

It was very quickly discovered that the term nephritis, qualified or unqualified, was a loose one, and was often used with such apparent lack of interest or understudy that very frequently there was no clear clinical data in the histories to warrant it. For this reason the number of cases which were really useful for investigation reduced itself to 100, and to obtain even this number it was necessary to include those in which the clinical history included such terms as "albumin present," "blood and pus," or "albumin and casts." Such expressions are obviously inadequate ones upon which to base a discussion, and yet if clinical diagnoses are made upon such fragmentary bits of information, and if histories are to serve their function in the present or in the future, then these inadequacies, because they indicate clinical historical incompleteness, should have attention attracted to them.

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\*From the Mary M. Emery Department of Pathology of the University of Cincinnati, and the Pathologic Institute of the Cincinnati General Hospital.



After a preliminary survey of these 100 cases they were divided into three groups, as follows:

- I. Those in which the clinical and anatomic diagnosis did not agree, 26.
- II. Those in which the clinical and anatomic diagnosis agreed, 36.
- III. Those in which there was no clinical diagnosis, but in which the clinical data indicated some renal abnormality, 37.

#### GROUP I.

In this group there was, as already noted, a discrepancy between the clinical conclusions and the anatomic findings. For instance, nine cases were clinically diagnosed as chronic diffuse nephritis, whereas at the postmortem table they proved to be acute. Seven were just the reverse, clinically they were acute nephritis; anatomically, chronic. In five cases the kidneys were tuberculous. The clinical diagnosis in two of these was acute nephritis; in two, chronic nephritis; and in one, cystitis. There were five other cases belonging in this group. One was diagnosed clinically as tuberculous nephritis and proved to be carcinoma of the kidney; two were clinically acute parenchymatous and anatomically diagnosed as "nephritis" and proved to be chronic diffuse nephritis.

The facts mentioned above are tabulated as follows:

TABLE I.

CLINICAL DIAGNOSIS.	ANATOMIC DIAGNOSIS.	NO. CASES.
Chronic diffuse nephritis	Acute nephritis	9
Acute nephritis	Chronic diffuse nephritis	7
Acute nephritis	Tuberculous nephritis	2
Chronic nephritis	Tuberculous nephritis	2
Cystitis	Tuberculous nephritis	1
Tuberculous nephritis	Carcinoma	1
Acute parenchymatous nephritis	Chronic diffuse nephritis	2
"Nephritis"	Chronic diffuse nephritis	2
Total		26

The five tuberculous kidneys are probably the most interesting and the urinalysis and clinical symptoms are worthy of especial attention. In the clinical cystitis case, there was a history of frequent and painful urination with a urinalysis of "S.g. 1010, albumin trace, few red blood cells, no pus or casts" and no other tuberculous lesion was found in the body. The two chronic nephritides showed urines with the following analyses respectively—S.g. 1024-1002, albumin trace, no cells or casts in either. The two acute nephritis urinalyses showed respectively S.g. 1020—not given, albumin neg.—a trace, few granular and hyaline casts, no blood—few red blood cells, and no casts. These last four cases had definite pulmonary lesions but no history of any urinary disturbance. The contrast between the two supposedly acute nephritic cases are typical examples of what one may expect to learn from urinalyses, as will be shown more fully in the course of this paper. Not only are they not the analyses of acute nephritis, but they mislead one as to the true condition existent.

The typical analyses referred to, which one ordinarily regards as definite, are, briefly mentioned: Urine in acute nephritis is decreased in amount, or com-

pletely suppressed, S.g. 1025 to 1028, sediment large, and albumin in large amounts, as high as 8.5 per cent. Microscopically there are usually red and white blood cells in variable amounts, epithelial cells, hyaline, blood and epithelial casts. The urine in the chronic diffuse nephritides with more of the interstitial change, shows an increased output, S.g. 1005 to 1012, very scanty sediment, albumin as a trace or completely absent, solids decreased in amount. Microscopically there is often a shower of red blood cells, fine and coarse granular or hyaline casts. A kidney of the parenchymatous variety of the chronic diffuse nephritis type, is expected to excrete a decreased amount of urine with a S.g. of 1020 to 1040, a heavy sediment, albumin present in large amounts  $\frac{3}{5}\%$  by weight. Microscopically there are hyaline epithelial, fine and coarse granular, and fatty casts. Leucocytes are abundant with occasional red blood and epithelial cells.

The above simple analyses only take into consideration those factors which are at every one's disposal and are more or less routine procedures in an institution.

In order to make this group more complete the urinalyses in the remainder of the cases are given as completely as they were in the history.

TABLE II.

URINALYSES OF CASES CLINICALLY DIAGNOSED CHRONIC AND ANATOMICALLY ACUTE.

S.g.	Albumen.	Casts.	Cells.
?* ?	Trace "	None ?	Reds and whites ?
1012	?	Very many cellular	Pus and reds
1022	+++	Granular	None
1020	Neg.	Hyalin and Granular	?
1018	"	Neg.	Reds
1020	+	?	?
1016	Neg.	Neg.	Neg.
1012	+	Granular	No Reds

\*?Not given in the history.

TABLE III.

URINALYSES OF CASES CLINICALLY ACUTE AND ANATOMICALLY CHRONIC.

S.G.	Albumen.	Casts.	Cells.
1022	+	Large amt. granular	?
?	++	?	?
?	++	Abundant granular	pus, no reds
		Epithelial, hyaline	
1040*	+	Granular	?
1032	++	?	?
1040	+	Granular, hyaline	?
1006	++	Granular, hyaline	?

\*Sugar present in urine.

The above analyses, incomplete as they are, are still sufficient to show how little one may depend upon an analysis in nephritic lesions.

## GROUP II.

In thirty-seven cases the clinical and pathological diagnoses agreed. The next question which arose concerning this group was, in how many cases where

he correct diagnosis was made did the analysis given correspond even fairly accurately to the composite description herein given? Only fourteen out of these thirty-seven cases showed the typical analysis which could warrant its being an aid in the diagnosis.

TABLE IV.

No. of clinical and anatomical chronic interstitial nephritis cases with typical urinalysis .....	5
No. of clinical and anatomical acute nephritis cases with typical urinalysis. .	6
No. of clinical and anatomical chronic parenchymatous nephritis cases with typical urinalysis.....	1
No. of clinical and anatomical pyonephrosis cases with typical urinalysis..	2

In the other cases the chief factor which was at variance was the specific gravity. Where given, it was almost invariably too high by quite a wide margin. In chronic diffuse nephritides the albumen was often marked three plus (+++) while in five acute conditions the only notice that was taken of albumin was the laconic expression of "present." Casts were too variable a quantity to deserve much mention. Coarse granular casts were found in the acute nephritides while in others no casts were found. The chronic interstitial varieties in most instances contained all varieties in considerable amounts.

In only a few of the sixty-three cases was albumin absent and yet its presence in large amounts was not an indication of the amount of renal damage. The converse proved to be equally correct.

As an example of the conditions frequently encountered, the following case covers a number of the points: In a fulminating case of lobar pneumonia the urine showed "S.g. 1020, albumin negative, a few granular and hyaline casts, and no abnormal cells." The clinical diagnosis was chronic diffuse nephritis, which corresponds very closely to the typical urinary picture except for the high specific gravity. Postmortem these kidneys proved to be involved in an extreme grade of acute nephritis, with no chronic change as one might expect from the clinical condition. This case is not an exceptional one but is a typical example of the discrepancies which one meets daily.

The high specific gravity in all cases would tend to mislead one did he not consider that the kidneys are only one exit for the elimination of water. In fevers with profuse sweating, in cases in which there is diarrhea, and in cases in which but little water is ingested, it is to be expected that the specific gravity would rise above that usually described in chronic nephritides. Further, the total solid excretion probably bears little if any relation to the water output, for in the functional renal test we often get a high percentage of phenolsulphonphthalein with a small amount of fluid excretion, while in diabetes with polyuria the specific gravity is high.

Also leucocytes or "pus" are not found where most expected. In five cases showing infarction, acute interstitial nephritis or "surgical kidneys" the urine was free from white blood cells, while in two others where the report was "quantities of pus" and the clinical diagnosis cystitis, the postmortem examination revealed pyonephritis.

It must not be considered that these comparisons are from picked or unusual cases. They represent typical clinical reports with their postmortem veri-

fications or contradictions. Of course, sixty-three cases may hardly be sufficient upon which to base a radical report, yet if one realizes that in at least five hundred and fifty out of the six hundred cases studied there was some nephritic lesion, and that of this number only sixty-three cases having both a urinalysis and a clinical diagnosis could be obtained, then it seems apparent that the clinicians also lay very little stress upon urinalysis as a diagnostic measure. So we are led to the conclusion that a urinary examination is of as little value, in itself, as a leucocyte count, a Wassermann, a gastric analysis, or other laboratory procedures without other data, and that in the differential diagnosis of kidney lesions the urinary picture is of practically no importance.

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## TECHNIC FOR STAINING BLOOD, ESPECIALLY FOR PLASMODIA

BY WM. KRAUSS, M.D., MEMPHIS, TENN.

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**S**TAINING technic. Dissolve 25 centigrams eosin, w.g. in 25 c.c. fresh distilled water. Label: No. 1. Dissolve 25 centigrams azur II in 25 c.c. fresh distilled water. Label: No. 2. Provide also chemically pure absolute methyl alcohol, fresh distilled water, anilin oil, each, a sufficient quantity; a 10 c.c. graduate; a Petri dish or special staining dish.

Note: The stains are best kept in glass stoppered "balsam bottles." These have "outside closure" and admit dropping pipets easily. "Ice house" distilled water is rarely fit for this purpose. It is well to have the special staining dish (Sargent) and the slide attachment for the centrifuge. (B. & L. O. Co.)

Proceed as follows: Flood the slide with the methyl alcohol. Measure 5 c.c. distilled water, add a drop of anilin oil, shake; add two drops No. 1, shake; add four drops No. 2, shake; pour into dish. After at least one minute's fixation in the alcohol, put the slides face down into the stain. The adhering alcohol may remain. Leave undisturbed for ten minutes. Remove slides, dry on centrifuge, examine under oil.

N. B.—Fungi may develop in the staining solutions and absorb stain. This necessarily changes the proportions; also, the droppers may be of unequal size. The proportion is right if the finished mixture is quite blue when seen in glass dish over white paper. The leucocytes must show very deeply stained, otherwise the parasites remain unstained. Never use the same dropper in both solutions, as they precipitate each other. To remove smudges and precipitates from the stained smear, gently wipe off with a pledget of cotton dripping wet with acetone, going over the slide *only once*.

# A CONVENIENT ELECTRICAL SUPPLY SYSTEM FOR A PHYSIOLOGY LABORATORY\*

BY CLAYTON McPEEK, M.D., C. I. REED, A.B., AND FRANK DECK,  
COLUMBUS, OHIO.

A CONVENIENT system of electrical supply is of considerable importance in a physiological or pharmacological laboratory. In most schools, the dry cell is still employed as a means of supply for use in experimental work. While this affords an independent supply for each student, or each group of students using the same set of apparatus, there are the disadvantages of constant expense in the purchase of new cells, time loss on the part of the student in securing and connecting new batteries, the clumsiness and inconvenience of the apparatus when the batteries are connected, and the constantly varying supply of current from this source.

On account of these disadvantages a new system has been installed in this laboratory, which, after several months of trial in class work has proved so satisfactory that it has seemed desirable that it be brought to the attention of others who may care to profit by its use.

The apparatus consists of a motor generator charging set, manufactured by the Robbins-Myers Co., of Springfield, O., which may be operated by either an alternating or direct current at either 110 or 220 volts. This apparatus generates 10 amperes at 8 volts D. C., charging a three cell storage battery of the "Ever-ready" type. This battery is of 6 volt, 80 ampere-hour capacity and is mounted on a shelf under the switchboard. On the switchboard are placed fused switches, jacks, fuses and parallel and series bar connections. The switchboard is made of a 1-inch marble slab, 12 inches by 24 inches, supported by iron brackets so as to leave a 5-inch space between it and the wall for making final connections after it is in place. The connections may be traced out by reference to the diagram in Fig. 1.

The 110 volt supply enters through the fused switch S, then passing to the motor end of the generator through the rheostat R, by means of which the speed and proper charging rate for the battery may be regulated. The current from the generator, at 7 to 8 volts, passes through the fused switch C.

When the battery is to be charged, both switches are closed. The current will flow from the positive (+) terminal of the switch C to the positive terminal 6, through the fuse and jack G to one cell of the battery; from the negative (-) pole of the cell to terminal 5, through the series bar B to terminal 4 and through jack F to the middle cell of the battery to terminal 3 and again through the series bar B to terminal 2 thence through the fuse to the jack E, and the remaining cell of the battery to terminal 1, thence to the negative side of switch C.

When the charge is completed, switch C should be opened first, otherwise the battery will discharge into the generator, since all jacks, terminals, fuses and switches are back-connected on the switchboard.

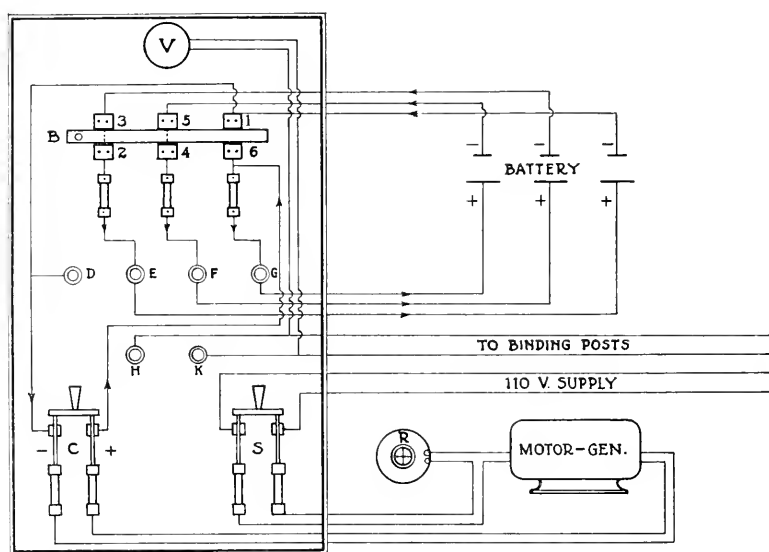
Jacks D, E, F, G, H and K are made of brass of  $\frac{5}{8}$  inch diameter, turned

\*From the Laboratory of Physiology of the College of Physiology of the Ohio State University.

down in a lathe, with a head, threaded to  $\frac{1}{2}$  inch and drilled out having an opening of about  $\frac{1}{4}$  inch in diameter. These are placed in holes drilled through the switchboard and held in place by nuts on the reverse side of the board. Under the nuts are brass clips for the connections with the circuit wires.

Terminals 1, 2, 3, 4, 5 and 6 are of spring brass 1-16 inch thick and 1 inch wide, bent into right angles and fastened to the board with brass machine screws which pass through the marble and support brass clips to which wires are soldered for the rear connections of the terminals. The two series of the terminals are  $\frac{5}{8}$  inch apart; into this space is inserted the series bar, used in charging or discharging the battery.

The series bar is of hard wood  $\frac{1}{2}$  inch thick with brass contact plates 1-16 inch thick, mounted on each side, corresponding to the positions of the ter-



minals. Copper rivets form connections between 2 and 3 and also between 4 and 5. Contacts 1 and 6 are not so connected. A  $\frac{1}{4}$  inch hole in one end of the bar fits onto a brass stud of corresponding size, mounted on the board, so that there can be no error in inserting the bar.

The parallel bar is also of hard wood, with contacts of brass mounted on each side so as to connect in parallel, terminals 3, 5 and 1 and also 2, 4 and 6. When the battery is not in use, the parallel bar is inserted. This permits of an "evening-up" between the cells of the battery so that all may be kept at, approximately, the same voltage. This bar may also be used when only two volts are required on the circuit, so bringing an equal load upon all the cells.

With this arrangement, three voltages may be supplied to the circuit H-K which leads to the binding posts on the desks in the laboratory. The connections between the jacks and the laboratory circuit are made by means of two cables about 18 inches long whose wires, passing through wooden handles at each end, are soldered onto brass plugs attached to the end of the handle. The plugs are  $\frac{1}{4}$  inch in diameter and slotted so as to form good connections when inserted into the jacks.

If one cable connects D to H and the other connects E to K, with the series bar in place, two volts are discharged. With D and H still connected, if K is inserted in F, 4 volts will be obtained. With the connections between D and H, and G and K, 6 volts will be discharged. If all the cells are not needed, and it is desirable to employ 4 volts, the load may be shifted by connecting E to H and G to K.

In charging the series bar is always used. Periodic readings of the specific gravity of the fluid in the cells are taken by means of a special hydrometer designed for the purpose. Whenever the gravity falls as low as 1.150, the battery should be charged until it reads 1.270. A fall in the voltage discharge as indicated by the readings of the voltmeter, V, which is tapped onto the circuit, indicates recharging. Each cell usually registers 2.1 volts. Charging can usually be done outside of the regular laboratory periods. Unless it is unavoidable, the cells should not be charged while the students are using the current for the generator will produce variations in the strength of the current that sometimes seriously disturb the experiments in progress.

Should it be desirable to test the voltage of the cells separately, the voltmeter may be detached from its connections with the laboratory circuit and applied to each cell.

The laboratory circuit is conducted by wires led through conduits under the floor and brought up to binding posts mounted on wooden blocks between the ends of the desks so that the students at each desk may have free use of one set of binding posts. A circuit from an electric time clock is also led through the same conduit and brought up to a separate set of posts mounted on the blocks between the desks.

Incidentally, it may be mentioned that pipes for gas and compressed air are brought up from the floor between the desks and fitted with two way outlets, so that each desk is supplied with gas, air, electricity and time current, independently. This arrangement removes the necessity of overhead wires or piping, which are both inconvenient and unsightly.

## NUMBER OF KEYS IN CIRCUIT.

## DISCHARGE VOLTAGE AVAILABLE.

	2.1	4.1
1	2.0	3.7
2	1.9	3.3
3	1.8	3.0
4	1.7	2.75
5	1.6	2.62
6	1.6	2.5
7	1.55	2.42
8	1.5	2.4
9	1.45	2.3
10	1.42	2.21
11	1.4	2.12
12	1.32	2.05
13	1.3	2.0
14	1.28	1.9
15	1.2	1.85
16	1.1	1.78
17	1.02	1.63
18	.9	1.54
19	.8	1.43
20	.76	1.2

In order to determine the efficiency of the system, a series of tests were made, in which the students were instructed to connect the inductoria with the binding posts at the desks, with a simple key in circuit. A voltmeter was connected with one pair of binding posts and readings were taken as the keys were closed, one at a time. Tests were made with line supplies of 2 and 4 volts. The results are shown in the preceding table. The first column shows the number of keys closed; the second shows the voltmeter readings with a line discharge of 2.1 volts; the third column shows the voltage with a battery discharge of 4.1 volts.

It will be apparent from this table that there is considerable variation in the line voltage when a large number of inductoria are in use at the same time. This disadvantage may be obviated if the battery is so placed in the laboratory that several feed lines may be leading off from it to the desks, thus placing only a few desks on one line. On account of the arrangement of this laboratory, it was found necessary to place the battery in a small room from which only one supply line could conveniently be led into the laboratory, hence all of the 32 desks are tapped onto one line. However, since but few of the students are using the current at the same time, the system is still more satisfactory, even under these conditions, than the old method of obtaining current from dry cells.

At first, there was installed a generator without the storage battery, which supplied current as needed. It was found, however, that this caused a vibration in the current in the primary coils of the inductoria sufficient to induce a very weak faradic current in the secondary coil. This interfered seriously with the efforts of the student to obtain satisfactory results on simple make and break stimulations. This also constitutes a disturbing factor if the battery is charged when the students are using the current. A series wound motor-generator can be used with more satisfaction than a shunt wound one.

Aside from this one disadvantage of variability in voltage, which does not become a disturbing factor under most conditions of laboratory experimentation, this system presents a number of advantages. The entire first cost of installation did not exceed \$125.00, and the expense of current and upkeep is very small. There is also a great saving of time to both the student and the instructor. There is a fairly constant supply at all times and the outlets are so distributed that the student can move his apparatus to any part of the laboratory and still be within easy reach of any set of binding posts. There is also a minimum amount of time required on the part of the student with relation to accessory apparatus, no part of the system requiring a great deal of attention in order to keep it in good condition and ready for use.



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## EDITORIALS

### *Tuberculosis*

THERE is a demand on the part of the intelligent laity that the crusade for the restriction of tuberculosis be pushed with all the intelligence and enthusiasm that the medical profession can command. The verdict of science is that tuberculosis is a preventable disease; one which we can reasonably hope to wholly eradicate in time from the list of diseases that afflict the race. The intelligent laity accepts this verdict and demands that the medical profession follow the way pointed out by science. Thirty years ago a sanitarium devoted to the care of tuberculosis patients was a rare thing. There were not many medical men at that time who had the visions which Trudeau saw in the forests about Saranac. Now, there are hundreds of sanatoria devoted to this disease. Nearly every state has one or more such institutions and municipalities are doing even better. Forty years ago the best physician was not sure that anyone ever recovered from pulmonary tuberculosis, his patient must show marked emaciation, a chronic cough, profuse expectoration, and an afternoon elevation of temperature. Practically all who reached this stage died and the conclusion generally reached by the laity from this disease. Before he could make an unquestionable diagnosis of pul-

best physicians was that pulmonary tuberculosis terminated fatally in one hundred per cent. This belief was quite universally held by the laity and the physician, who had a heart like other men, postponed announcement of his diagnosis, he equivocated, he temporized, and often he lied directly. At that time and under the conditions then existent, this was to his credit rather than otherwise, but he got into a habit which many older men have never been able to cast aside and more unfortunately still, many younger ones have acquired. In the sixties Villenin demonstrated experimentally that tuberculosis is an infectious and transmissible disease, but his work threw no light on the diagnosis, and while the up-to-date physician might take some precautions against the spread of the disease, he gained in nothing else. In the eighties Koch identified, isolated and grew in pure cultures, the infective agent, the existence of which in tuberculous sputum, Villenin had demonstrated. For a time after Koch's discovery the identification of the specific bacillus was deemed essential to a positive diagnosis. This was a step in advance, but a short one. If we wait until tubercle bacilli have appeared in the sputum, in the majority of instances we have waited too long. At present with marked advance in the interpretation of physical signs, by a close study of the clinical fluctuations in temperature, especially as influenced by exercise, by x-ray studies and by means of the different tuberculin tests, the disease can in most instances be recognized while it is yet in its incipency and while it is highly amenable to hygienic treatment. Hundreds, and in some sections, thousands of people are now going to the physician to find out whether they have the infection. Forty years ago it was a cruel thing to pronounce a diagnosis of tuberculosis even when its existence in an advanced stage was plainly evident. Today failure to recognize this disease in its earliest stages shows ignorance on the part of the physician and may result in irretrievable injury to the patient.

The wide-awake, conscientious physician is on the lookout for every scientific advance in our knowledge of tuberculosis. Doctor Gerald B. Webb, of Colorado Springs, has consented to become a member of our editorial staff and will devote his energies to the subject of tuberculosis.

—V. C. V.

1915

# *The Journal of Laboratory and Clinical Medicine*

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NO. 3.

## ORIGINAL ARTICLES

### AN EXPERIMENTAL INVESTIGATION OF CERTAIN PHENOMENA RELATING TO THE ACTION OF DRUGS ON THE RATE OF OXYGEN CONSUMPTION IN THE ANIMAL BODY\*

BY D. E. JACKSON, PH.D., M.D., ST. LOUIS, MO.

A SIMPLE, cheap form of apparatus for recording the relative rates of oxygen consumption by an anesthetized animal is shown in Fig. 1. This apparatus is practically identical with that described in a recent article<sup>1</sup> on the employment of closed ether anesthesia for ordinary laboratory experiments. In fact I have used the same apparatus often for both purposes at the same time. For a detailed description of the construction of the apparatus (which costs but little and is intended primarily for *student experiments*) I must refer those interested to the former article. It will be seen from the illustration that a round disc of very thin aluminum (or pasteboard) has been attached (with mucilage) to the center of the upper surface of the rubber bath cap which covers the large pan into and out of which the animal breathes. From the aluminum disc a thread passes up over a pulley and thence to a second pulley placed above the frog heart lever which writes on the drum. The thread is attached to this lever by a bull dog clamp which serves also as a counterpoising weight to counterbalance the two or three bull dogs which usually have to be placed on top of the aluminum disc to cause it to descend readily as the animal inspires. The method by which a record of the oxygen consumption is obtained on the drum is shown in the figure. This record has a saw-tooth-like appearance. The narrow, almost perpendicular lines (which should normally all be of the same length) are made by the writing lever when oxygen is run into the pan. The drum should be running at a very slow speed (or even stopped for the few seconds required for the oxygen to run into the pan) when these lines are made. The broader, ascending curves represent two things, first the amount of air which actually passes into

\*From the Department of Pharmacology of Washington University Medical School.

and out of the lungs at each inspiration and expiration. A contraction or dilatation of the bronchioles will, of course, show its effects immediately on this curve. And second, the curve forms a record of the relative rate of oxygen consumption by the animal. Both of these points are often of great interest in studying the action of drugs. It will be noted that the oxygen is run into the pan only at intervals and the signal for its admission is the arrival of the broad ascending curve to the same height as that which the previous saw-tooth had. Just enough oxygen is then quickly injected to bring the writing lever down to the former low point. Thus the height of each saw tooth should be practically the same.

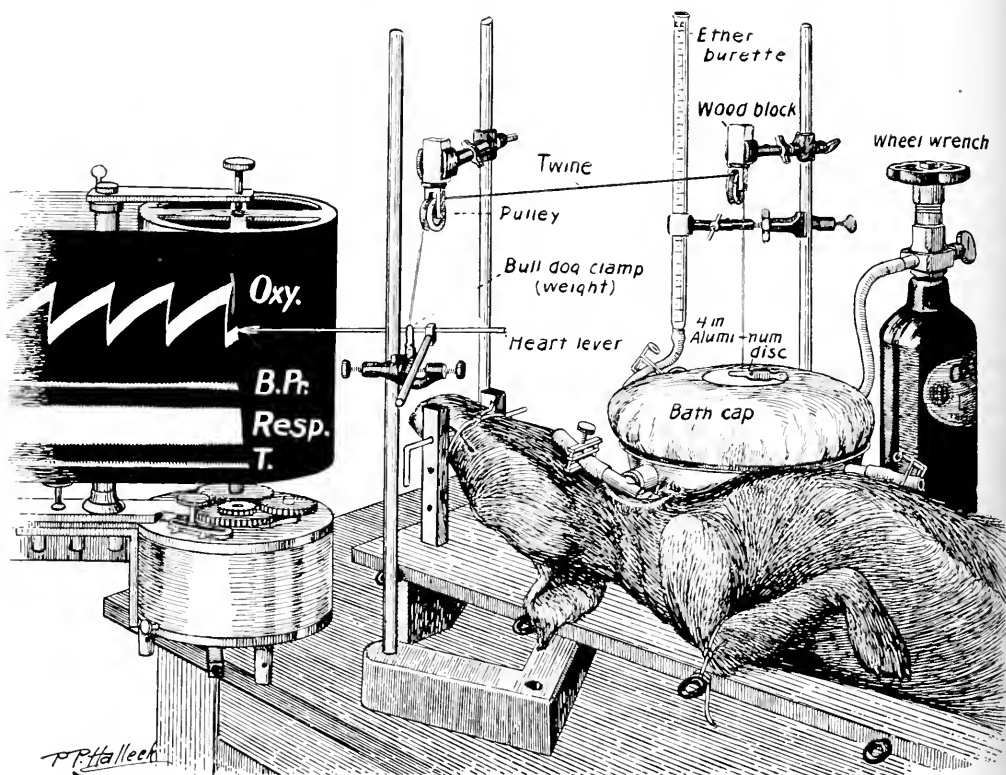


Fig. 1.

This is a relative matter which can be set arbitrarily to suit the operator for any given experiment or for any sized dog.

The distance between the descending lines marks the relative amount of oxygen which the animal consumed during that period of time (recorded in seconds, etc., on the drum). For example if in one three minute interval the animal consumed a given amount of oxygen and the first saw-tooth was made to correspond with this, but in the second three minute interval the dog consumed only half as much oxygen then the distance covered by the second saw-tooth will be twice as great as that covered by the first.

Special emphasis must be laid on the fact that the animal is excreting carbon dioxide all the time and this carbon dioxide is being continually absorbed by the strong sodium hydrate solution in the bottom of the large pan. The rate

of this absorption varies but little and can not interfere with the correct interpretation of large variations in the rate of oxygen consumption. Small variations in this rate which may be quickly compensated for by the animal may apparently often be somewhat obscured, especially when observations extend over only a brief period of time. But if a longer period is used then any sudden large excretion of carbon dioxide will be absorbed and the relative amount of oxygen consumed in the period will be shown on the record. This equalizing influence of longer periods of time seems to hold true for temperature (and perhaps for water and ether vapor variations) also.

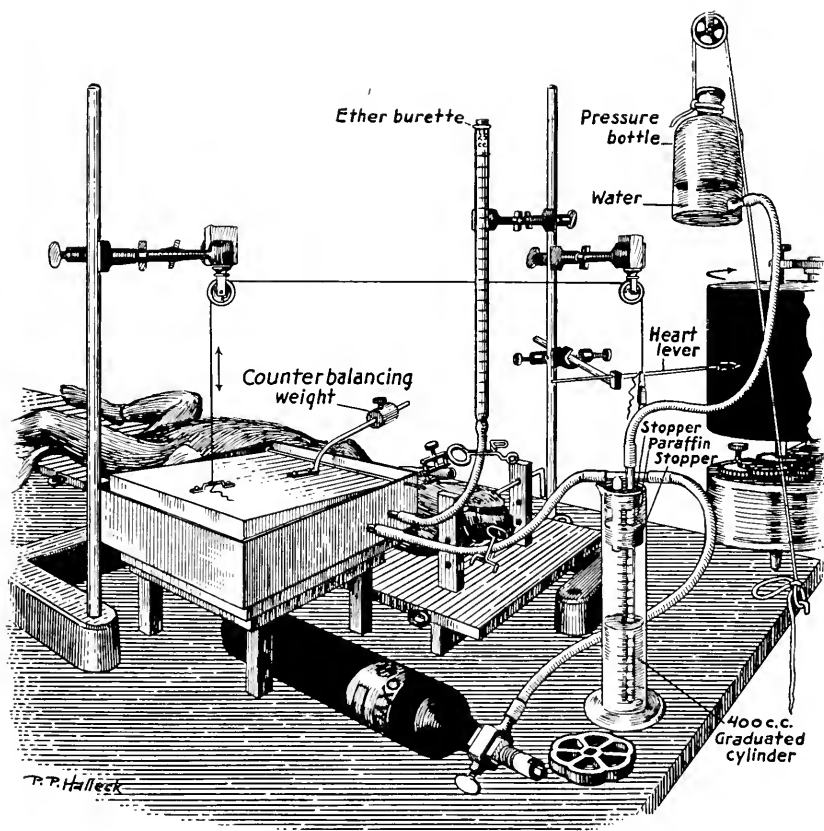


Fig. 2.

A better but somewhat more complicated form of the apparatus is shown in Fig. 2. Here a large, shallow, square pan is used as an absorption chamber. The details of the construction of this pan are shown in Figs. 3 and 4. A breathing pan made of exceedingly thin aluminum or brass (the latter is preferable) is hinged at the back and can dip up and down in a trough of water which fills the space between the outer wall of the large pan and a second inner wall which is soldered at the bottom to the floor of the large outer pan. This inner wall forms a second pan within the large outer one. Strong (not saturated) sodium hydrate solution is placed in the inner pan to the depth of about one inch. The breathing pan then is lowered and dips into the trough of water all

around thus forming an air-tight chamber into and out of which the animal breathes through the large spout at the back. The tracheal cannula (side tube) is passed through a large cork which is inserted into the spout. The breathing pan is carefully balanced on the hinges by an adjustable counterbalancing weight. The apparatus is thus made very sensitive and the mechanical hindrance to the breathing of the animal is reduced to a minimum. As shown in Fig. 2 a twine string is attached to the top of the breathing pan. This string

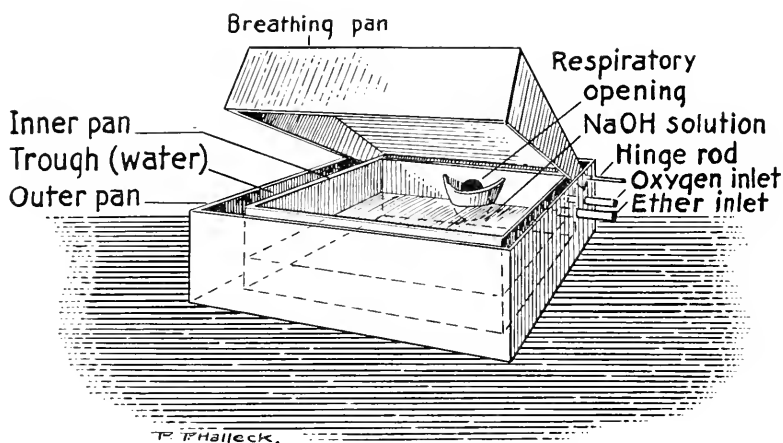


Fig. 3.—Interior view of the apparatus shown in Fig. 2. The "hinge rod" can be pulled out endwise to free the "breathing pan" which can then be entirely removed.

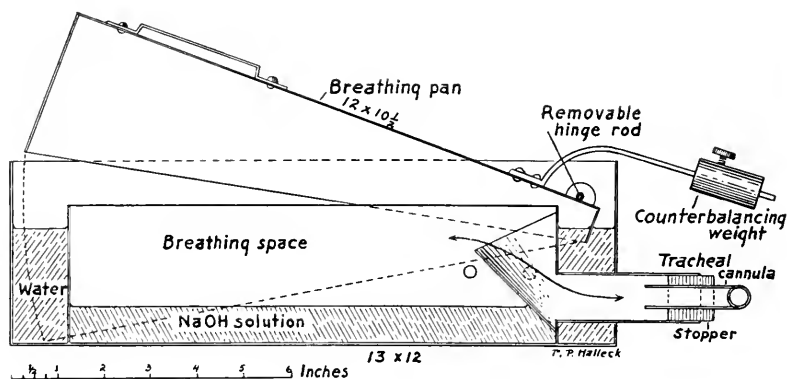


Fig. 4.—A longitudinal cross-section through the middle of the apparatus showing details of construction.

passes over two pulleys and finally is clamped with a bull dog forceps in the proper position to the long arm of a heart lever which writes on the drum.

An oxygen measuring device is also shown in Fig. 2. Oxygen under pressure is injected from a tank into the graduated cylinder through a T-tube the side tube of which passes through the two corks and a layer of paraffine which seals the upper end of the cylinder down to the graduation marks. The remaining straight end of the T-tube connects to a rubber tube which is ordinarily closed by a spring clip but which when open conducts the oxygen into the breathing pan. A pressure bottle containing water is suspended above the apparatus. A

tube leads from the pressure bottle down to the graduated cylinder where it is connected with a small glass tube which passes through the corks and paraffine and reaches down to the bottom of the graduated cylinder. Within the cylinder about 100 to 300 cubic centimeters of water is placed. If the oxygen tank be opened a little, oxygen will be forced at once into the top of the graduated cylinder. The water in the lower part of the cylinder will be forced up into the pressure bottle. Thus the cylinder can be quickly filled with oxygen. The tank valve is then closed and the charge of oxygen remains stationary in the cylinder waiting to be run into the breathing pan. This is done by simply opening the spring clip in the tube between the cylinder and the pan. The water in the pressure bottle will at once run down into the cylinder and force the oxygen over into the pan which will rapidly rise. On the drum this would be indicated by the abrupt descending limb of the saw-tooth record. The amount of oxygen thus run into the pan at each emptying of the cylinder can be only approximately measured on the cylinder because the glass tube inside passes down through the cylinder, and the water remaining in the tubes, etc., still further changes the cylinder readings as does also the pressure variations. The cylinder therefore is used only for approximate estimations. The exact measurement of the amount of oxygen in each charge (which is constant and is determined by the amount of *transferable water* placed in the cylinder) is obtained by filling the cylinder with oxygen (or air), then disconnecting the inlet rubber tube from the pan and collecting from this tube under water in a second graduated cylinder the oxygen (or air) as it is discharged from the first cylinder on opening the spring clip. By the use of this automatic measuring device oxygen can be quickly and accurately run into the breathing pan and always under the same pressure. And in making the records the greatest difficulty which one experiences is in determining just at what moment the writing lever has reached its former high point on the drum. This is best done by placing the writing pointer of a stationary tambour or signal magnet in such a manner that as the drum turns this pointer will mark a line on the surface of the drum just at the level of the apex of each saw-tooth in the record. This stationary writing point should be placed just in front (to the right on most drums) of the recording heart lever. By watching the ascent of the lever the observer can then come very close to determining the correct moment to inject a new charge of oxygen. (I unfortunately did not do this until after I had used the method for some time.) If there is an error in the time selected for the injection, however, this will be detected at once when the oxygen is run in for the lever will not descend to its former low-level line, but will either be below (injection made too soon) or too high (injection made too late). Thus a double check on each injection can be at once obtained. An obscuring influence which is sometimes present is a marked change in the depth of respiration of the animal. With drugs which cause these changes the high point on the record is sometimes difficult to determine. With strychnine, e.g., this point is hard to make out at the top of the record when convulsions come on but with a little experience one can soon make a very close approximation. Since each saw-tooth should cover only one to three minutes and the amount injected at one time can be checked up immediately with the following saw-

tooth, small variations due to respiratory disturbances can only cause errors for a brief time.

The form of anesthesia used with this apparatus is a matter of some concern. For ordinary purposes when dogs are used a small dose of chloretone is advisable. In addition to this a little ether, perhaps two or three cubic centi-

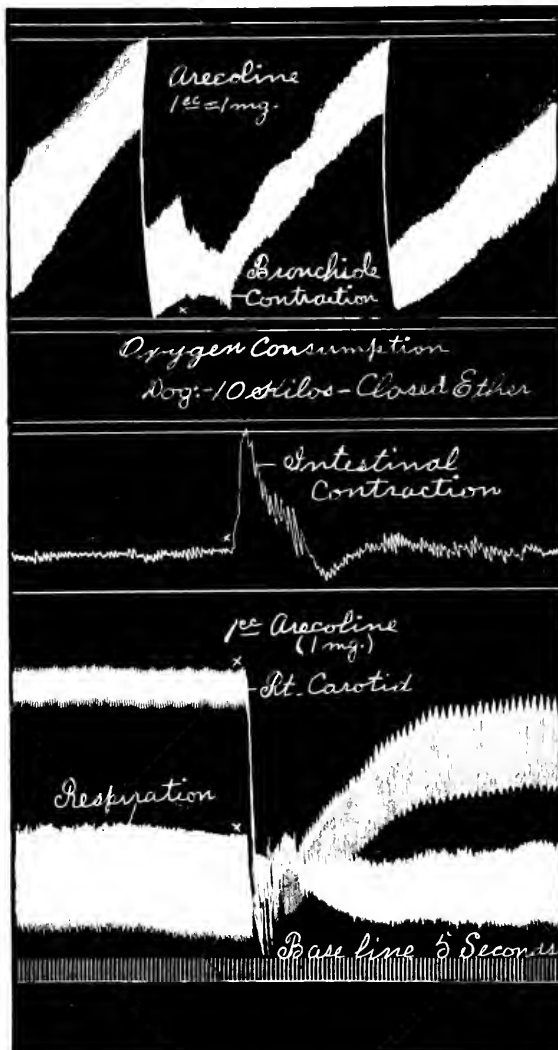


Fig. 5.

meters, may be injected (from a burette) into the pan. A perfectly even regular anesthesia should then be maintained. Ether alone may be used but there is a slight source of error in this since when the amount of oxygen in the pan is small (pan down) the concentration of the ether vapor breathed may be greater than when more oxygen is present (pan up). This might be sufficient to obscure some of the readings. In a few experiments I have used the apparatus for spinal



dogs. To do this I have used a special piece of apparatus<sup>2</sup> which I inserted into the chest (to hold the walls open and rigid) and then negative (interrupted) pressure was used to aspirate the chest at regular intervals. Artificial respiration was thus carried on after the animal's brain was destroyed. Perhaps this method might be used to investigate a number of problems concerning metabolism in animals in which the central nervous system plays only a minor role, or perhaps takes no part at all if both brain and cord are destroyed.

There are a few sources of error which may be present in this method. As indicated in the article referred to above<sup>1</sup> some carbon dioxide accumulation

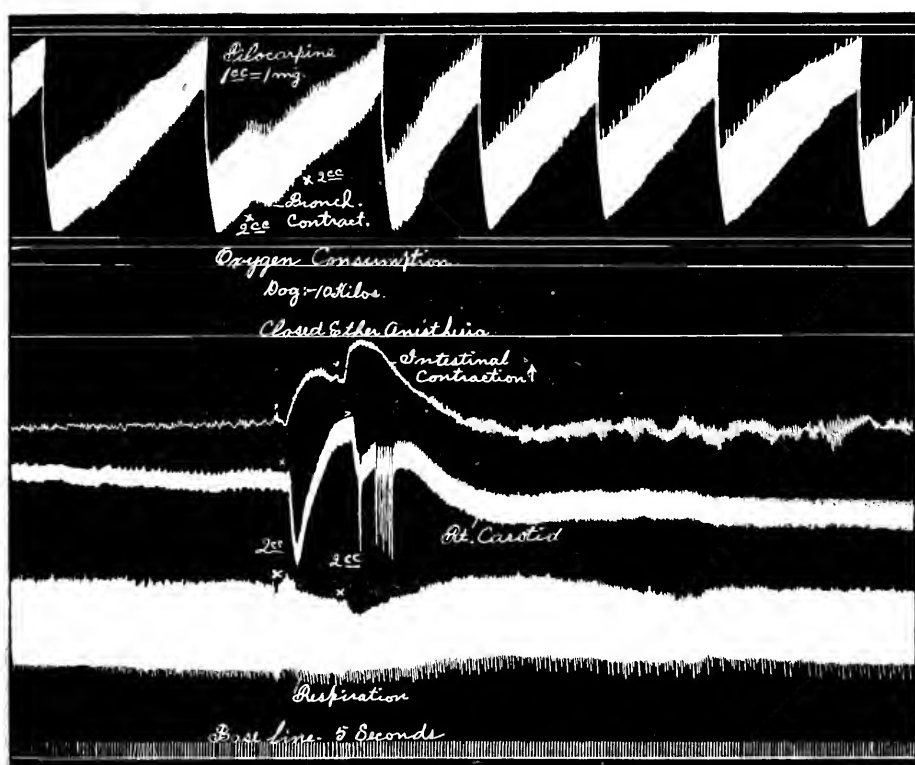


Fig. 6.

may occur in the breathing pan. With a strong fresh solution of sodium hydrate (*which must always be cold when it is used*) this does not appear to cause any practical disturbance provided the absorbing surface of the hydrate solution is great enough (large pan). A cold solution in the pan, cold water in the trough (Fig. 2) and the cold metallic walls of the pan (or the thin rubber bag in the apparatus shown in Fig. 1) serve to counteract the rise in temperature which the respired air might otherwise undergo. The careful counterbalancing of the breathing pan (or of the weight of the aluminum disc on the bath cap) together with the large, free, open spout through which the animal breathes serves to reduce to a minimum any mechanical obstruction to the respiration.

There are some interesting and obscuring influences which certain drugs

develop within the animal itself, however, and while these may sometimes interfere with the use of this method for determining the rate of oxygen consumption, the method, on the other hand may be used to great advantage for studying these very phenomena.

Among these drug reactions should be mentioned first of all perhaps the sudden increase in the rate of carbon dioxide excretion which a rapid increase in the rate and depth of respiration may bring about. In a brief period of time



Fig. 7.

this excess of  $\text{CO}_2$  will affect the record but after a time the sodium hydrate solution appears to catch up in its rate of absorption. Again if the animal suddenly begins to breathe deeply the expired air may be warmer (more expanded) than before and an excess of watery vapor may be thrown off. A large pan and plenty of cold solution, etc., will overcome this also when the respiration of the animal returns to normal. Again the injection of a drug which is wholly or partly excreted by the lungs (e.g., sodium sulphide, after which small amounts of  $\text{H}_2\text{S}$  escape in the breath) as a gas or vapor may affect the record. The in-

jection of a small amount ( $1/2$  c.c.) of ether from the burette into the pan may affect the record at once, especially if the temperature of the room or pan is moderately high. Presumably this would also be noticed after the intravenous injection of a solution of ether, although I have not yet tried out the experiment.

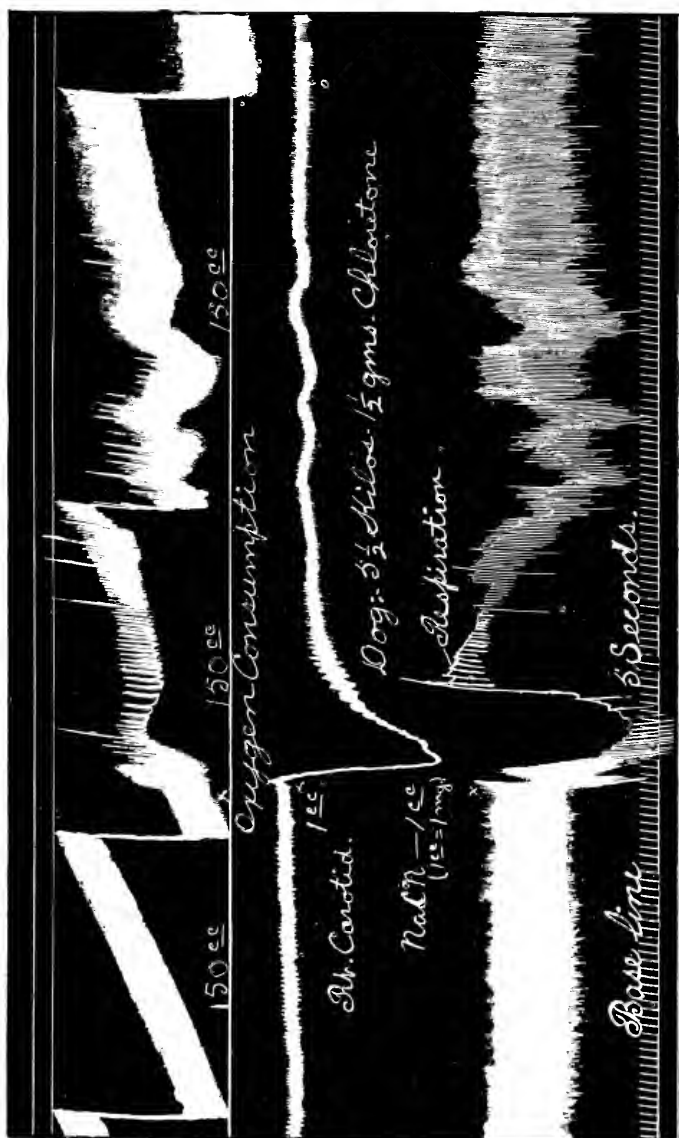


Fig. 8.

The gradual lowering of the vitality of the animal (perhaps from the prolonged anesthesia) in a long experiment may also show as a gradual progressive reduction in the amount of oxygen consumed per minute.

Fig. 5 illustrates a peculiar phenomenon brought out by drugs which cause marked constriction of the bronchioles. From this tracing it will be seen that

immediately after the intravenous injection of one milligram of arecoline hydrobromide there was a sudden drop in blood pressure (stimulation of the inhibitory endings of the vagi in the heart) and a marked decrease in the amplitude of the



Fig. 9.

respiratory movements (tracing marked "respiration"). A brief increase in the intestinal contractions also occurred (vagus stimulation). The point which is mostly to be emphasized here, however, is the change in appearance of the oxy-

gen consumption record. The ascending curve of this tracing was suddenly decreased in amplitude (less air passing into and out of the lungs). This change might be due wholly or in part to a depression of the respiratory center, thereby causing a decrease in the respiratory efforts. It is not at all probable, however,

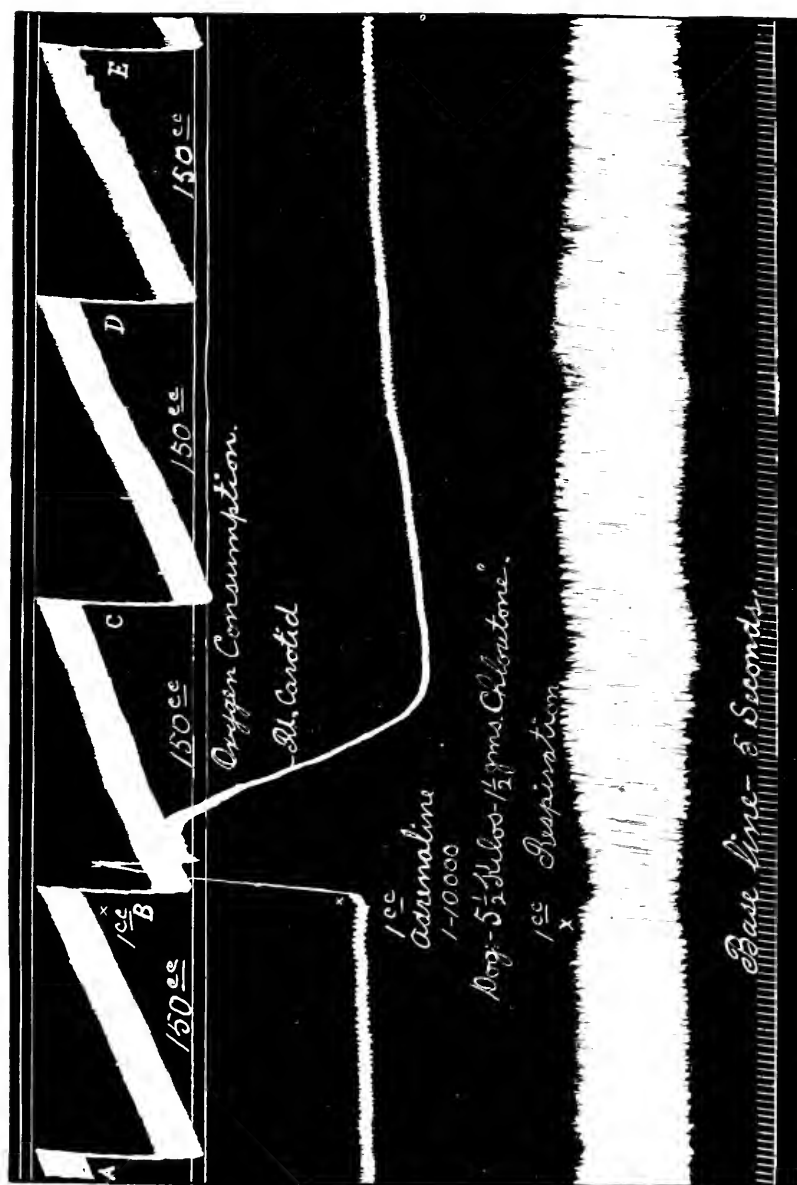


Fig. 10.

that this is the main cause for it can easily be shown experimentally that this dose of arecoline will greatly constrict the bronchioles and this lessens the volume of air which the lungs will hold. It seems evident therefore that this constriction of the bronchioles is mainly responsible not only for the decrease in

amplitude of the oxygen tracing, but also for the actual fall of the ascending limb of the oxygen record. This action of arecoline does not last long, however, and after a brief period the oxygen record again begins to ascend in the usual fashion. These results correspond exactly with the action of arecoline on the bronchioles. And this sudden change in the ascending limb of the oxygen record might be used as a method for studying the action of broncho-constricting drugs. It is possible that this intense broncho-constriction may hinder the absorption

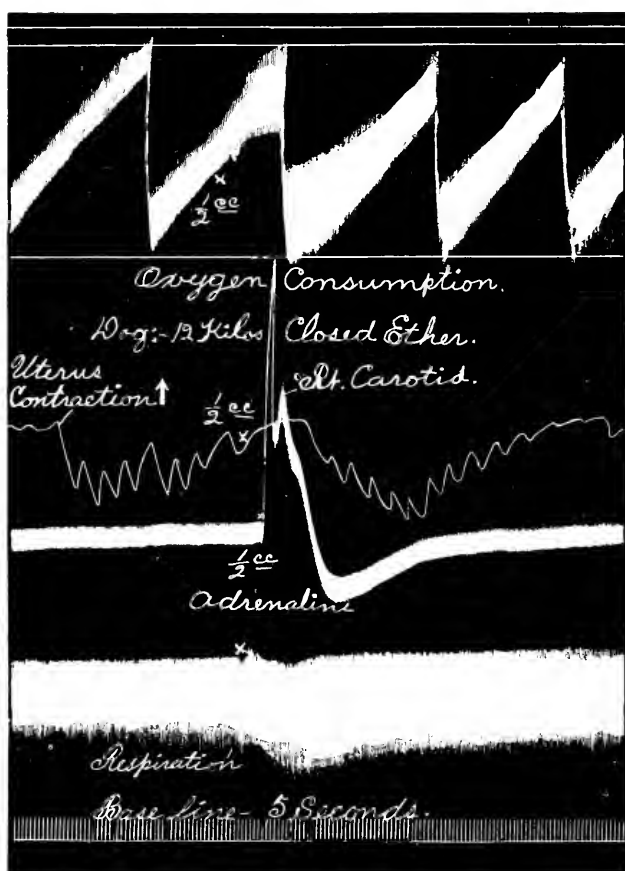


Fig. 11.

of oxygen or the excretion of carbon dioxide by the lungs, but the record does not perhaps show any such change in this case. And on the basis of other experiments (with different drugs), and since the animal was breathing a very high percentage of pure oxygen I am inclined to suspect that the broncho-constriction did not appreciably affect the gaseous exchange so far as the *mechanical actions* were concerned. This point is of interest in consideration of the dyspnea present during the paroxysmal periods of bronchial asthma. Fig. 6 shows the action of four milligrams of pilocarpine (in two doses) injected intravenously into the same animal. Here the broncho-constriction was distinctly present but less marked than after the arecoline. But following the

administration of the drug there is a very evident change in the rate of oxygen consumption which is increased. Possibly this is due to the great increase in the flow of the secretions as well as to the contractions of certain groups of smooth muscle which followed the injection of the drug. In this case although there is a moderate prolonged broncho-constriction produced by the drug there is evidently no lessening of the rate of oxygen consumption. This again is of interest in consideration of the dyspnea present during the paroxysmal periods of bronchial asthma.

In reference to these two records a further point should be mentioned. A number of observers, especially Haldane and Smith,<sup>3</sup> and Bohr<sup>4</sup> have suspected that gaseous exchanges at the surface of the lungs may be partly controlled by some secretory mechanism which may possibly be under nervous control. And Dreser has shown that in fishes pilocarpine will cause an increased secretion of oxygen into the swimming bladder. If either of these drugs possess any such action as is here indicated on either the absorption or excretion of oxygen or carbon dioxide by the lungs of dogs the records obtained by this method might be influenced by this action.

An interesting phenomenon is shown in Fig. 7 in which a dose of "ergamine" ( $\beta$ -iminazolyethylamine) was injected into a dog. A marked broncho-constriction must have been produced by the drug and this usually persists for a considerable length of time. The evidence of the constriction is seen on the oxygen record while the marked and prolonged fall in blood pressure shows that the drug was producing its usual effects. In spite of these changes, however, the rate of oxygen consumption undergoes but little change from the normal. This again reminds one of the changes in bronchial asthma. In this tracing the blood pressure fell to approximately one-third the normal level (mercury manometer) but this had only a minor influence on the oxygen record.

Figs. 8 and 9 show the action of sodium cyanide and morphine respectively on the rate of oxygen consumption. By comparison it will be seen that while in the cyanide tracing the blood pressure remained well up to the normal (after the primary fall), in the morphine record the pressure was only about one-half as high as it had been before the drug was injected. Furthermore the rate and depth of the respiration was more affected (in the latter part of the tracing) in the morphine experiment than in the cyanide record. Yet the changes in the relative rate of oxygen consumption were fairly comparable in the two cases. A comparison of Figs. 6, 7, 8 and 9 at once indicates that the rate of oxygen consumption in the animal organism is not only influenced in very different ways by various drugs but that within fairly wide limits the rate of absorption of oxygen by the lungs (and presumably the elimination of carbon dioxide) is largely independent of the height of the blood pressure, of the rate and depth of the respiratory movements and of the contraction or relaxation of the bronchioles. With reference to cyanides I may add that very small doses do not appreciably slow the rate of oxygen consumption, but on the other hand they appear, at least in some instances, to actually accelerate the consumption of the gas.<sup>5</sup>

In Figs. 10 and 11 I want to call attention briefly to a phenomenon which I have observed a great many times although in other instances I have failed to

obtain it. This consists in what appears to be a brief slowing down of the rate of oxygen consumption immediately after the intravenous injection of a fairly large dose of adrenaline. A large number of investigators have discussed the action of adrenaline on the metabolism of the body, especially in connection with the relation which the drug may have to the production of glycosuria, or to the oxidation of carbohydrates in the body. Among these may be mentioned Blum,<sup>6</sup> von Noorden,<sup>7</sup> Wilenko,<sup>8</sup> Edmunds,<sup>9</sup> Lusk,<sup>10</sup> Macleod,<sup>11</sup> and many others. I am inclined to believe, however, that none of these investigators have had in mind exactly the point which I mention here. It will be observed from the tracings that the first one (or two) saw-tooth notches following the injection (*intravenous*) of a good sized dose of adrenaline are longer than either the normal notches or the notches which follow later after the action of the drug wears off. While I have sometimes failed to obtain this result still I believe that under perfectly satisfactory conditions, and when the animal is strong and vigorous, this result should always be produced. I need not attempt any explanation of the phenomenon for the present. The vascular constriction over a considerable part of the body for a brief period might cause such a result. And the dilating action which the drug exercises on the bronchioles may also be concerned in some way for this dilation passes away as the action on the blood pressure ceases. It appears that the period of slower oxygen consumption (if the phenomenon is really due to this) corresponds more directly with the period during which the action of the drug is passing off, i.e., while the blood pressure is falling and for a few moments thereafter. Cannon<sup>12</sup> and his co-workers have recently emphasized the action which adrenaline has on muscular contraction and on the stimulation threshold of fatigued muscle. I should not be surprised if in the long run the phenomena observed by Cannon and his associates are found to be rather intimately related to the action of adrenaline on oxygen consumption which I have here indicated.

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# STUDIES OF THE TISSUE REACTIONS TO VARIOUS PRODUCTS OF THE TUBERCLE BACILLUS.\*

BY PLINN F. MORSE, M.D., AND ETHEL STOTT.

THE histological structure of the tubercle is unique in that it is almost the only specifically characteristic tissue reaction to injury. Hosts of injurious agents cause lesions and tissue reactions more or less alike. The streptococcus, the colon bacillus, and various chemical substances all cause tissue reactions grouped under the head of various inflammatory phenomena but the specific presence of none of these agents can be affirmed or denied on the grounds of the tissue reaction alone. Even the spirocheta pallida, while its various lesions are often characteristic and capable of positive diagnosis from their histology, many times causes lesions not at all typical enough histologically to make them positively diagnosable. Lacking the organism itself, in the picture, as in actinomycosis or blastomycosis, or some product of its growth as the cyst of echinococcus, the histologist has practically two infectious conditions which he can positively diagnose from the appearance of the tissue reaction, namely, tuberculosis and leprosy.

The interest in this point of view was stimulated by our receiving in the laboratory some tissue from a patient who had had her face injected with paraffin for prosthetic purposes after mechanical injury. There appeared at the site of injection, lumpy tumors in the areolar subcutaneous tissue. These were removed and we were impressed by the fact that they were strikingly like young tubercles in structure. They consisted of avascular epithelioid granulations containing giant cells. Discussion of the subject with Dr. V. C. Vaughan, Jr., recalled the fact that he had held for a long time that the tubercle was the product of a foreign body reaction and not a reaction to a living organism primarily. This suggested the relationship of paraffin to the tubercle bacillus in that they were both waxy bodies; and this work was undertaken to study these reactions.

## THE LITERATURE.

The mechanism of the formation of the tubercle has been a subject for investigation for a long time. Wyssokowicz,<sup>1</sup> cited by Masur,<sup>2</sup> found that tubercle bacilli were present and stainable in the tissues as long as one month after the injection and that epithelioid giant cells were grouped around them in the liver. Koch<sup>3</sup> found that dead tubercle bacilli caused aseptic pus upon subcutaneous inoculation. Buchner<sup>4</sup> believed that the albuminates of the bacteria were responsible for the lesions. Mafucci<sup>5</sup> did a large number of experiments inoculating killed tubercle bacilli subcutaneously with the result that the animals died several months later with a general marasmus, atrophy of all organs with stasis of lungs, liver, kidneys and spleen. There was a large quantity of pigment in the spleen. While Mafucci ascribed these deleterious results of the injection of dead tubercle bacilli to toxic

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substances, Prudden and Hodenpyl<sup>3</sup> worked with cultures which were carefully washed and then heated for a long time in water or five per cent glycerin. The dead bacilli were then freed from all water-soluble metabolic products. Subcutaneous injection caused aseptic pus, and intraperitoneal injection caused nodules on the serous surfaces similar to miliary tubercles but no caseation took place. When intravenously injected a part of the animals died of malnutrition in the third week and the rest died after longer periods. After three or four days, the bacilli were evenly distributed throughout the organs, lung, liver and spleen. From the fifth day on there formed in the lungs numerous white nodules which microscopically were typical of tubercles and contained bacilli which stained well. Similar pictures were found in the liver. Four to six weeks after the inoculation, the lungs of the animals were exactly similar to those in which virulent bacilli had been introduced. The bacilli began to disappear in the later weeks and became granular. They obtained like results by spraying the trachea with dead cultures.

From these findings, Prudden and Hodenpyl concluded that apparently the protoplasm of tubercle bacilli contained poisons which cannot be extracted by the usual methods but which are set free in the animal body and act with positive chemotaxis on the animal tissue causing the formation of the nodules.

Straus and Gamaleia<sup>7</sup> record substantially the same results as Prudden and Hodenpyl, finding that dead tubercle bacilli could form perfect tubercles.

Only once in the literature am I able to find the idea specifically stated that the tissue reaction to the tubercle bacillus is that of a foreign body. Vissman,<sup>8</sup> while agreeing in the main with Prudden and Hodenpyl, and with Straus and Gamaleia in regard to the lesions produced, showed that these lesions were the same whether the bacteria were killed in their own water of condensation or by heating in Ehrlich's fuchsin solution and comes to the conclusion *that the tubercle bacillus is a powerfully stimulating foreign body*. Like Prudden and Hodenpyl, Vissman was not able to produce caseation.

Masur<sup>2</sup> reviews the previous literature and sums up the whole matter in the following conclusions:

1. Dead tubercle bacilli injected intravenously cause either a severe general disease or death of the animal.
2. There are constantly found in injected animals, tubercle-like nodules in the lungs with giant-cells but without caseation, further, a more or less severe chronic interstitial pneumonia.
3. A part of the animals used show catarrhal pneumonia processes in the lungs, inflammatory changes of varied sort in the kidneys, pigment deposits on the spleen and disease of the vessels.
4. It is not important in what manner the bacilli are killed.
5. The organ changes as a whole, are apparently dependent upon the influence of toxic substances which are liberated by the disintegration of the tubercle bacillus.

Abel<sup>9</sup> believes that a body was set free from the bacteria by the tissues which acted as an irritant and caused the tubercle.

Gamaleia<sup>7</sup> occasionally obtained some caseation and believed that caseation did not occur because the animals died too quickly. He believed that the

bacilli, in addition to their chemotactic effect on the body cells, contained a destructive poison.

Grancher<sup>10</sup> showed that the effect of injected killed avian tubercle bacilli in rabbits was to cause typical peritoneal tuberculosis.

Koslenitsch<sup>12</sup> showed that injection of sterilized tubercle bacilli into the anterior chamber of a rabbit's eye either caused pus or after four to eight weeks, nodules in the iris which consisted of epithelioid tissue and a few giant cells. The nodules did not caseate and contained bacilli.

Weyl<sup>13</sup> was apparently the first to attempt an analysis of the effects of chemically separate constituents of the dead bacilli. He obtained two bodies from the tubercle bacillus, one of indifferent nature which had the tinctorial properties of the bacteria and the other a toxomucin which upon subcutaneous injection caused dry necrosis of the skin.

Grancher and Martin<sup>14</sup> found in their efforts to immunize animals, that after injection of very old cultures of tubercle bacilli the animals frequently died of parenchymatous nephritis. This result, as well as the occasional appearance of paraplegia and cachexia was ascribed to a toxic substance in the body of the bacillus.

It is evident from these references that the preponderant opinion of investigators has been that the histological lesion caused by the tubercle bacillus was due to a poison liberated from the body of the bacillus by action of the tissue cells. To test these ideas, the bacilli were grown in mass culture on ten per cent glycerin bouillon in three or four liter Erlenmeyer flasks. After a thick pellicle had formed the flasks were autoclaved and the growth obtained by filtering off the bouillon and washing the residue with cold water. The sterile growth was dried over sulphuric acid in the desiccator and then ground to a powder. The powder was extracted in two fractions. Ether was first added and the mixture kept in the ice box for twenty-four hours and then the ether filtered off. Evaporation of the ether extract yielded a thick brown oil which soon became green by exposure to light and air. The residue after ether extraction was dried in air and extracted in a reflux condenser in boiling alcohol for one-half hour, filtered hot and the filtrate evaporated. The residue consisted mostly of protein germ substance and remains to be investigated.

The evaporated filtrate from the boiling alcohol extract yielded a light yellow wax-like substance darkened slightly to a reddish-brown on standing (impurities?). These two lipid substances were tested separately for their effects upon rabbits, and white rats. Rats were injected with quantities varying from 0.25 to 1.0 c.c. intraperitoneally and in the groin. The animals were killed at intervals of three days to four weeks after injection by putting them in a small chamber and turning in illuminating gas. They stopped breathing within thirty seconds, although the heart was usually beating at the autopsy.

The dark, viscous oil extracted by cold ether produced no lesions at any time. It was injected intraperitoneally and subcutaneously in the rat's groin and after the initial inflammatory reaction had subsided the tissue was normal again. Intravenously in rabbits, doses short of the production of severe pulmonary embolism produced no lesions whatever.

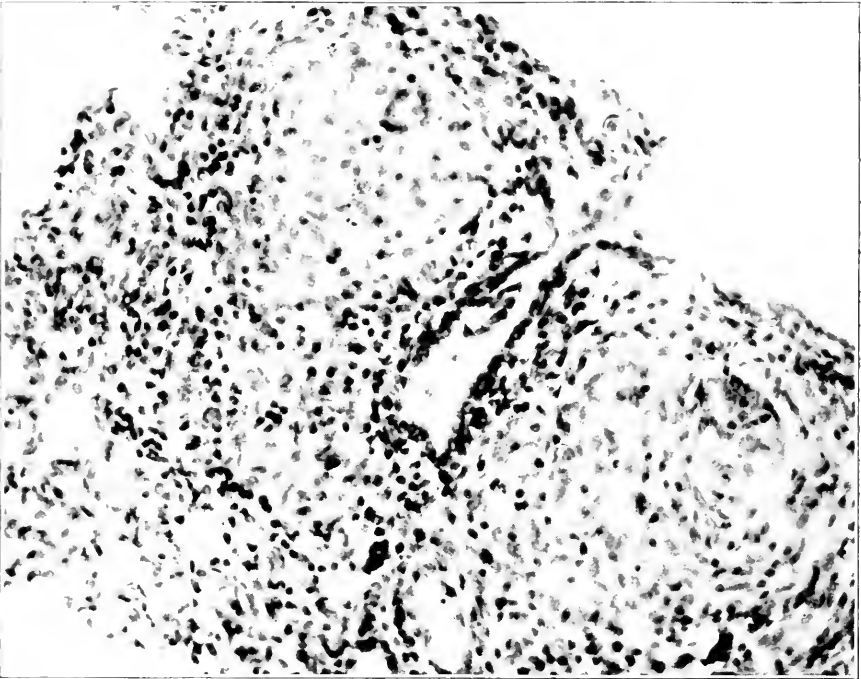


Fig. 1.—Rabbit's lung four weeks after intravenous injection of wax from tubercle bacillus. Epithelioid tubercles with giant cells.

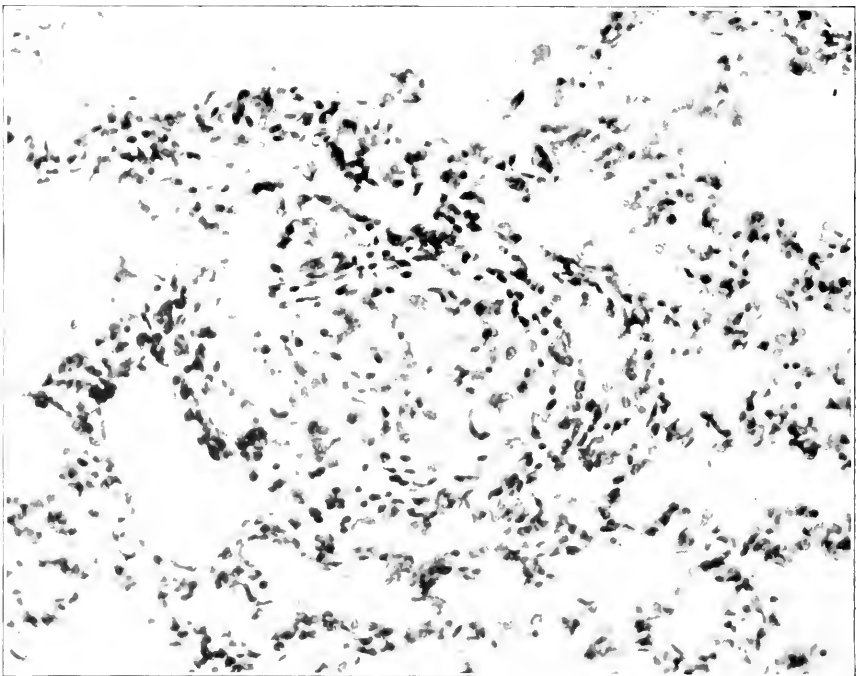


Fig. 2.—Epithelioid tubercle four weeks after intravenous injection of waxy substance.



Fig. 3.—Epithelioid tubercle with giant cells in rabbit's lung four weeks after intravenous injection with waxy substance from tubercle bacillus.

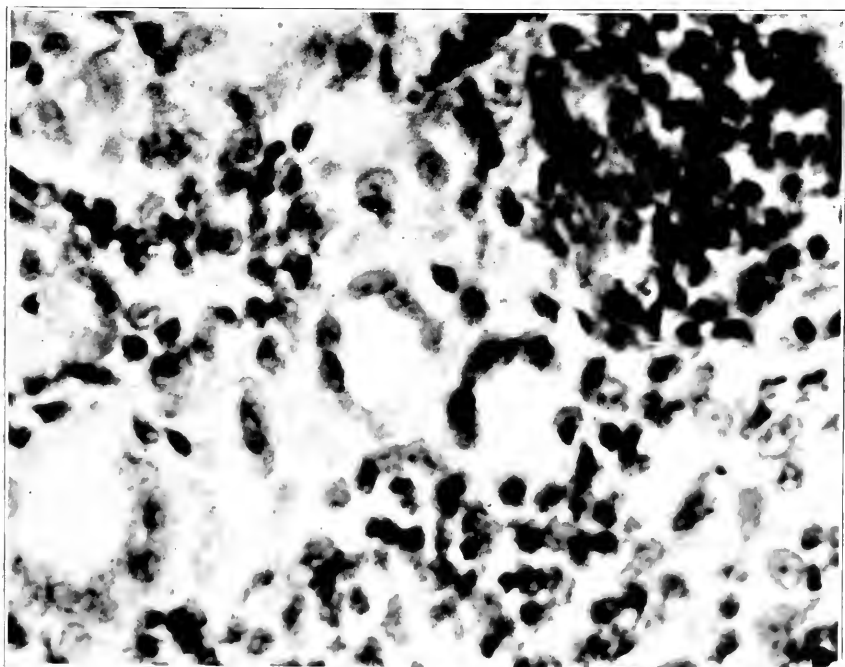


Fig. 4.—Giant cell formation in epithelioid lesion due to waxy substance from tubercle bacillus.

Effects produced by the waxy substances can be divided into early and late lesions. The wax was injected in doses of 0.25 to 1 c.c. in the same manner as the oily substance. The initial vascular reaction was not great, but in about twenty-four hours the site of injection was found to be indurated and swollen and if opened at this time, a serous exudate, containing many eosinophile cells, had formed around the wax droplet.

The substance was introduced into the lung of the rabbit both dissolved in olive oil and warmed until fluid enough to flow through a syringe. In all cases, the wax was sterilized carefully and injected aseptically. Most of the

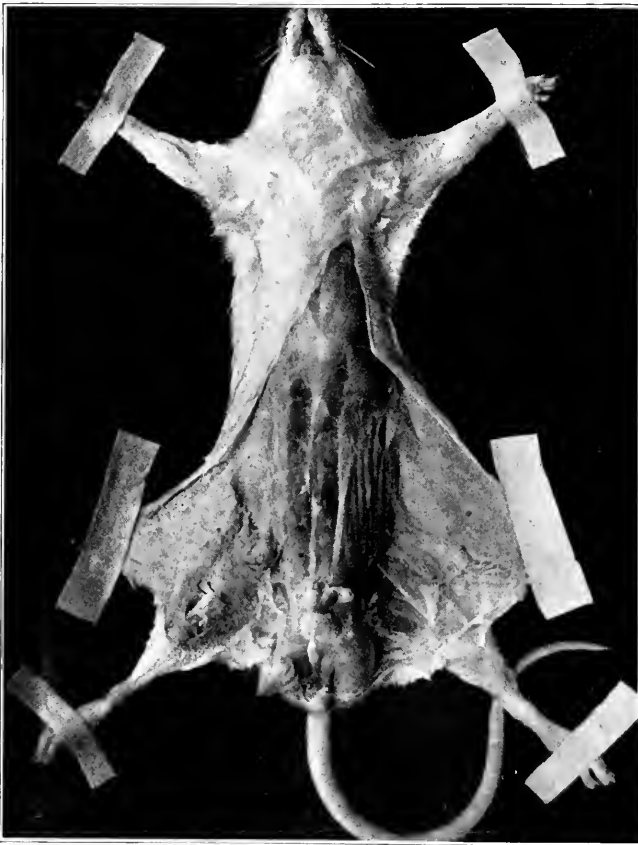


Fig. 5.—Nodules in right groin two weeks after subcutaneous injection of tubercle waxes.

substance lodged in the capillaries near the outer margins of the lung in the lower lobe as in experimental fat embolism. At times, where a large dose was given too rapidly, a large enough embolus was introduced to produce infarction of a portion of a lower lobe; usually, however, a few hours after injection examination showed only intense edema and blanching of the margins. In twenty-four hours polymorphonuclears were abundant and the tissue blazed with eosinophiles when stained with hematoxylin and eosin. The wax appeared not to produce any marked degree of thrombosis. Once in a lesion, three days' old with a good sized infarct, an induced thrombus was observed.

The lesions in the lung were most interesting at the end of three days. By this time, endothelial proliferation was very active and whole areas of the lung tissue were filled in solid with endothelium, polymorphonuclears, and eosinophile leucocytes. There was considerable fibroblastic reaction also (Fig. 8, A). The endothelium on the capillaries which were distended by the wax first swelled up and projected into the lumen soon obliterating the capillary and forming a syncytial mass with many dividing endothelial cells (Fig. 8, A and B). This method of occlusion of capillaries is interesting in view of the fact that it gives a possible explanation of the avascularity of a tubercle.



Fig. 6.—Intraperitoneal lesions two weeks after injection of tubercle waxes.

The usual method of vascular obliteration assumed heretofore has been that the growing bacillus quickly injures the endothelium of the capillaries in the infected area, leading to thrombosis with subsequent organization. This view does not seem justified inasmuch as the peculiar type of granulation tissue characteristic of tuberculosis is avascular from the start and consists primarily of endothelial whirls. I believe that whatever tendency to the formation of vascular capillary buds there may be is subverted by the unusual stimulus driving the endothelium to proliferate, thereby obliterating the endothelial cords and changing them to a syncytial mass (Fig. 8, A).

The development of the whirls of the endothelial cells now progresses until the wax is entirely walled off and the lesion reaches its full development in about four weeks (Figs. 1, 2, 3, and 4).

There were found in the subcutaneous lesions, lumpy masses filled with yellow purulent material surrounded by avascular epithelioid granulation tissue containing giant cells. The contents of these softened areas were never caseous but either purulent or filled in solid in the smallest ones with granulation tissue of the characteristic type (Fig. 5).

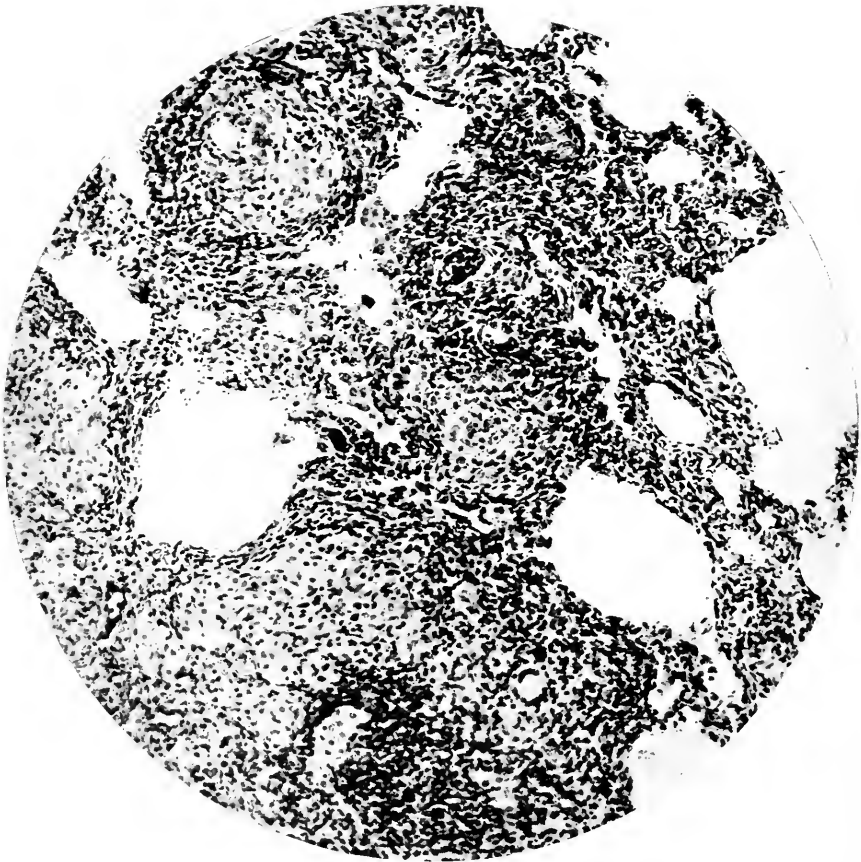


Fig. 7.—Low power. Rabbit's lung four weeks after intravenous injection of wax-like substance of tubercle bacillus.

In the peritoneum (Fig. 6) the lesion presented a typical appearance at the end of two weeks. The omentum was stretched transversely in a roll below the stomach or twisted and adherent to various portions of the abdomen. There were numbers of little white spots like miliary tubercles which microscopically were found to consist of epithelioid whirls without blood vessels. The surface of the liver showed tiny white points and the lower margin was covered by an organized exudate.

The resulting picture in the rabbit's lung, after four weeks, was most constant (Figs. 1, 2, 3, 4, and 7). All the signs of acute inflammation were gone.





Fig. 8.

- A. Endothelial syncytium. Showing dividing endothelial cell and cosmophiles. Early lesion.  
B. Capillary after injection of wax-like substance showing beginning endothelial obliteration of vessel.  
C. Late lesion. Avascular epithelioid tubercle four weeks after injection of wax like substance.



The leucocytes and eosinophiles had almost disappeared. Scattered irregularly throughout the lung were typical structures consisting of avascular epithelioid whirls of granulation tissue (Figs. 2, 3, and 7). There was an increase of connective tissue in the lung as a whole, evidenced by the thickening of the septa. The areas did not undergo caseation and were well walled off by young fibroblasts (Fig. 8, C), as one would expect in a non-progressive condition. At this time, giant cells were numerous (Figs. 3 and 4) formed by fusion of the epithelioid cells. The wax had not yet disappeared, but was still present in giant cells and in tissue spaces surrounded by avascular granulation.

The animals never showed cachetic phenomena or other abnormalities through the experiments. The wax is apparently totally devoid of toxic properties. Whether either the oily substance or the wax is a single substance or a mixture of several substances has not been determined. The word wax has been used entirely in the physical and not in the chemical sense throughout this article.

These results have suggested the problem of more careful chemical separation of all the constituents possible from the tubercle bacillus with separate testing of their tissue reactions.

The literature on the chemical side of the subject is not given here since it does not pertain to this mechanical portion of the "Arbeit." In these lesions, like all those produced by the whole dead bacillus caseation never occurred. Whether actual necrosis can be accomplished in this way or not is doubtful. The ability to cause the tissue to die is possibly an attribute of the living bacillus. The role of the possibly unsaturated fatty substances of the bacillus in preventing the digestion of the dead tissue by leucoproteases as suggested by Petersen and Jobling is a promising subject for investigation.

The frequency of appearance of tubercles in organs having a large amount of endothelium in their structure, such as lymph nodes, lung, liver and spleen, may be something more than a mechanical coincidence. Klotz<sup>11</sup> has shown that olive oil used as a menstrum for cholesterol injections has a tendency to cause selectively a proliferation of the endothelium.

It seems clear, however, from this short series of experiments, that the tubercle bacillus produces a microscopically characteristic lesion not because it is a living particulate protein, but because its waxy substances act as a peculiar type of foreign body.

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## TONSILLECTOMY DURING THE COURSE OF ACUTE RHEUMATIC FEVER.\*

BY ROGER S. MORRIS, M.D., CINCINNATI.

THERE is a considerable group of workers who hold that the tonsils should be removed as a routine procedure in all cases of chronic infectious arthritis, in which the origin of the infection is at all obscure. The law of probabilities is here relied on, for it is the experience of many that the tonsils harbor the focus of infection more often than other parts of the body. And it is probably safe to conclude that all who painstakingly follow this rule in chronic infectious arthritides have seen brilliant results, not in all cases nor even in a majority of them, but nevertheless in a sufficient proportion to justify the procedure. Even when the operation fails to bring the hoped for relief, we have no proof, in the absence of animal inoculations, that it was not indicated, for the work of Rosenow and others has shown the frequency with which multiple foci of infection are encountered. So, too, many of us are having tonsils removed in cases of recurring endocarditis, myalgia, and so on.

The present paper deals, however, not with tonsillectomy in chronic infectious arthritis, but with its value in acute rheumatic fever, a field in which, so far as I am aware, the indications for the procedure, its dangers, and the possible benefits to be derived from it are not so generally appreciated. Our own experience is limited to a small but encouraging group of cases.

It is a generally accepted clinical fact, first noted by Eyerlen<sup>1</sup> in 1798, that acute rheumatic fever is very frequently preceded by acute tonsillitis. The association is of such common occurrence that for years the tonsils have been looked upon as one of the portals of entry of the infection. Not only in primary attacks of acute rheumatic fever, but also in subsequent attacks, it is a common experience to obtain a history of sore throat or acute tonsillitis as the initial event in the illness. The arthritis or endocarditis, chorea or erythema nodosum then follows in a comparatively short time after the onset of the throat symptoms. In other words, it is often evident that the rheumatic cycle begins with manifestations of tonsillar infection.

The frequency with which acute rheumatic fever is preceded by sore throat is variously estimated. Pribram<sup>2</sup> finds it in only 1.5 per cent of cases. Dieulafoy<sup>3</sup> says it occurs in at least a third of all cases. Others find evidence of tonsillitis, acute or chronic, in as much as 80 per cent of the cases.

It is evident from a perusal of the literature and also from clinical experience that the history given by the patient is often unreliable. The local symptoms in the throat are so mild oftentimes that little attention is given to them. Furthermore, there may be serious disease in or about the tonsils, with little or no local manifestations. Simple inspection of the throat may not be sufficient in many cases to detect the trouble. It is significant that recent writers, employing more thorough methods of examination of the tonsils, find these organs diseased

\*From the Cincinnati General Hospital.

as a rule in a much higher percentage of cases of acute rheumatic fever than was formerly the case.

It is not presumed, of course, that the tonsils are the only portal of entry or depot of infection in acute rheumatic fever. Indeed, there is good clinical evidence to the contrary. Abrasions in the mucosa of the nose have been followed by acute polyarthritis with or without endocarditis in a number of instances. Such cases are recorded, among others, by M. Senator,<sup>4</sup> de Stella, and von Kronenberg. Goadby<sup>5</sup> and others report cases of acute polyarthritis originating from pus pockets about the teeth. Mackenzie<sup>6</sup> believes the virus may gain access to the body of children at times by way of the bronchial or intestinal mucosa. But all are apparently agreed, at the present time, that the most frequent portal of entry and focus of infection is to be found in the faucial tonsils.

Given, now, a case of acute articular rheumatism, there can be no hesitation regarding the proper medicinal therapy, i. e., salicylic acid. Fortunately or unfortunately, the joint symptoms of the great majority of cases respond promptly to this drug or its derivatives. But the question naturally arises, is one's duty to the patient discharged when the acute arthritis has been controlled? Is the patient, in other words, fully protected by the relief of his symptoms—his joint pains? It is universally known, of course, that such is not the case. In no disease are complications of commoner occurrence than in acute rheumatic fever.

Osler's<sup>7</sup> statements regarding the frequency of complications in rheumatic fever give a good general idea of their prevalence, though there is probably considerable deviation from the general averages at times, endocardial infection, for example, being apparently much commoner some years than others. Endocarditis, resulting eventually in chronic valvular disease, occurred in more than 50 per cent of 889 cases analyzed by Church,<sup>8</sup> while it was found in about the same proportion of 116 of Mackenzie's cases in the first attack, in 71 per cent during the third attack. Romberg found 58.5 per cent of 670 cases of endocarditis attributable to rheumatism.

Pericarditis, while not unusual, is met with much less often than endocarditis.

Myocardial changes are almost inevitable with either peri- or endocarditis.

Pneumonia and pleurisy occur in about 10 per cent of cases.

Other complications are rarer. But it is clear that while salicylic acid is usually efficacious in relieving the arthritis, it is practically powerless in controlling the infection in other parts of the body.

It is such considerations as these which have led many to look upon the complications not as such but rather as parts of the disease picture. 'To Gürich,<sup>9a</sup> I believe, belongs the credit of emphasizing the probability that in acute rheumatic fever joint symptoms and the so-called complications are all the result of metastatic infections from a primary focus elsewhere in the body. And if one is to accept this conception of the disease, the therapy can no longer consist merely in the administration of salicylates, the application of local treatment to the joints, and rest in bed.

The first to attempt systematic treatment of acute rheumatic fever through the tonsils appears to be Gürich,<sup>9a</sup> who, in 1904, reported seventeen cases. Twelve of his patients had more or less severe tonsillitis, and four had peritonsillar abscesses preceding the appearance of the acute arthritis. In fourteen of the

cases plugs were seen in the crypts. He cites a few illustrative cases of acute rheumatic fever which were cured rather promptly by local treatment of the tonsils, but he does not say what proportion of the patients were benefited. Enucleation of the tonsils was not done.

In the following year, Gürich<sup>9c</sup> published a small monograph dealing with one hundred and forty cases of arthritic affections, subjected to the so-called tonsillar therapy. Fifteen of these cases are discarded, as the patients began treatment but left for one reason or another before the treatment was completed. Of the remaining one hundred and twenty-five cases, twenty-three were entirely unaffected by treatment of the tonsils, which consisted, as in his previous report, of making parallel incisions through the tonsils as early as possible, and within one to two weeks, curetting away what remained of the tonsils. Ninety-eight of the patients, he says, were finally cured by this treatment. His series, apparently, was not limited to cases of acute rheumatic fever but included also cases of chronic infectious arthritis. The proportions of these diseases are not stated.

In 1908, Rosenheim<sup>10</sup> reported ten cases of acute articular rheumatism in whom tonsillectomy was performed, two to six or more weeks following the onset of the acute arthritis. All were improved, the arthritis subsiding promptly in a number of instances. From the abstracts of histories, it appears that in no case did endocarditis develop subsequent to operation. Streptococci were found in all of the tonsils removed, in the majority of instances in pure cultures. No early operations were performed. As far as I can discover, Rosenheim is the first to enucleate the tonsils in acute rheumatic fever.

Hess,<sup>11</sup> in the following year, called attention to the frequency of tonsillitis in acute rheumatic fever. Acute follicular, phlegmonous or catarrhal tonsillitis or quinsy may usher in the attack. The author also emphasizes the importance of chronic inflammation of the tonsils in the etiology of the disease. Hess does not resort to surgical treatment of the tonsils in all cases. He gives no statistics of his own experience.

Schichhold,<sup>12</sup> in 1910, reported a series of seventy cases of acute rheumatic fever treated by Gürich's method. In practically all of his cases, thorough examination of the tonsils showed evidence of disease. He called attention to the importance, too, of looking for other foci of infection, such as the sinuses or teeth. Schichhold made incisions in the tonsils, as a rule, and later removed them, using Gürich's method. Subsequent to the incision, there was often immediate improvement in the joints, with a return of the symptoms in two or three days,—a reaction which Gürich had noted. At the time the joint symptoms subsided, the temperature usually fell; there was then a rise for two or three days, followed again by a fall. The second operation caused little reaction. The author strongly recommends the tonsillar treatment of acute articular rheumatism. His impression is that recent endo- and myocarditides heal more quickly after the operation on the tonsils.

Curschmann<sup>13</sup> cites cases of septicemia and of acute nephritis secondary to tonsillar infections, but he believes Gürich and Schichhold go too far in saying

that practically all cases of acute recurring polyarthritis should undergo tonsillar treatment.

A few other case reports are to be found in the literature, which support the frequency of the tonsillar origin of acute rheumatic fever.

The tendency has been, I believe, to defer the operation until the patient has recovered from his illness—to perform what might be termed an interval operation, both as regards arthritis and tonsillitis. *A priori*, it would seem that there are certain dangers in deferring the operation. For, if the tonsils are the focus of infection, their presence means that there is always a possibility, in fact, a probability, of metastatic infections of the heart or, less often, of other organs. It would, therefore, appear that, in cases of acute rheumatic fever where there is a definite history of tonsillitis during the present or a previous attack of arthritis, or where the tonsils are found to be diseased, they should be removed as soon as the operation can safely be performed.

To determine whether—and when—to operate, a number of factors must be considered. The first and most important desideratum is the service of a skilled throat specialist, who shall not only perform the operation but also determine whether the acute process in the tonsils (if it be of recent date) has subsided sufficiently to make operation reasonably safe. Judgment is often difficult, as the occasional occurrence of endocarditis following tonsillectomy demonstrates. (Cases VII and VIII.\*) But from a limited experience, we feel that, with the subsidence of outward evidence of acute inflammation of the tonsils, the danger of allowing them to remain in the body probably exceeds the danger of their removal. In all cases, we believe, at the present time, that an attempt should be made to relieve the joint tenderness by full doses of salicylates, before the operation is attempted. A certain amount of freedom of movement is necessary after the operation, to enable the patient to expectorate, etc.; during the height of an acute arthritis, pain on moving would often prevent the necessary changes in position. The condition of the patient also determines the choice of anesthetic, and various factors again enable one to choose between general and local anesthesia.

The following brief abstracts of histories are cases in point:

#### CASE I.

F. B., hospital No. A-2009, a white male, single, aged 19, a furniture worker, was admitted to the North Medical Service of the Cincinnati General Hospital, March 20th, 1916, complaining of "rheumatism in the legs."

The family history was unimportant.

*Past History.*—The patient has had sore throat almost every winter for several years.

*Present Illness.*—Two days ago, the patient first began to suffer with pain in the legs and hips. He was unable to walk and was brought to the hospital in an ambulance.

*Physical Examination.*—The patient is a strongly built man. His tonsils are hypertrophied and reddened and the cervical glands are enlarged but not tender. The knees are swollen and show mottled erythema. There is exquisite tenderness in the knees. There is slight swelling of the ankles about the outer malleoli. The heart is normal.

The urine shows a faint trace of albumin and a few granular casts.

The Wassermann reaction is negative. Blood culture is sterile.

The temperature rose to 103 the day after admission.

\*It is quite possible that in each of these patients endocardial infection had occurred prior to operation, though definite physical signs of valvular trouble were not present until after operation.

The symptoms were relieved by salicylates and the temperature became normal on March 23rd. Tonsillectomy was performed on March 29th. There were no other joint symptoms. The patient was discharged well, on April 6th.

CASE II.

G. W., hospital No. 5876, a white male, single, aged 30, a keeper of a market stand, was admitted to the North Medical Service of the Cincinnati General Hospital on November 11th, 1915, complaining of "rheumatism."

Family history is unimportant.

*Past History.*—The patient has had sore throat in the past and the tonsils were "removed" about twelve years ago. The patient had his first attack of acute articular rheumatism eleven years ago. He was in bed seventeen weeks at this time and says that every joint in the body was affected except the spine. The arthritis began in the feet and worked upward until the jaws were involved. He has had no cardiac symptoms. He denies venereal infection.

*Present Illness.*—The onset was acute on November 6th, 1915, with pain and swelling of the ankles. Within the last four days the knees and shoulders have become involved.

*Physical Examination.*—The patient is a strongly built and well nourished man. Pyor-

CINCINNATI GENERAL HOSPITAL

NO. 5876 G.W ADMITTED Nov. 11, 1915 WARD B III

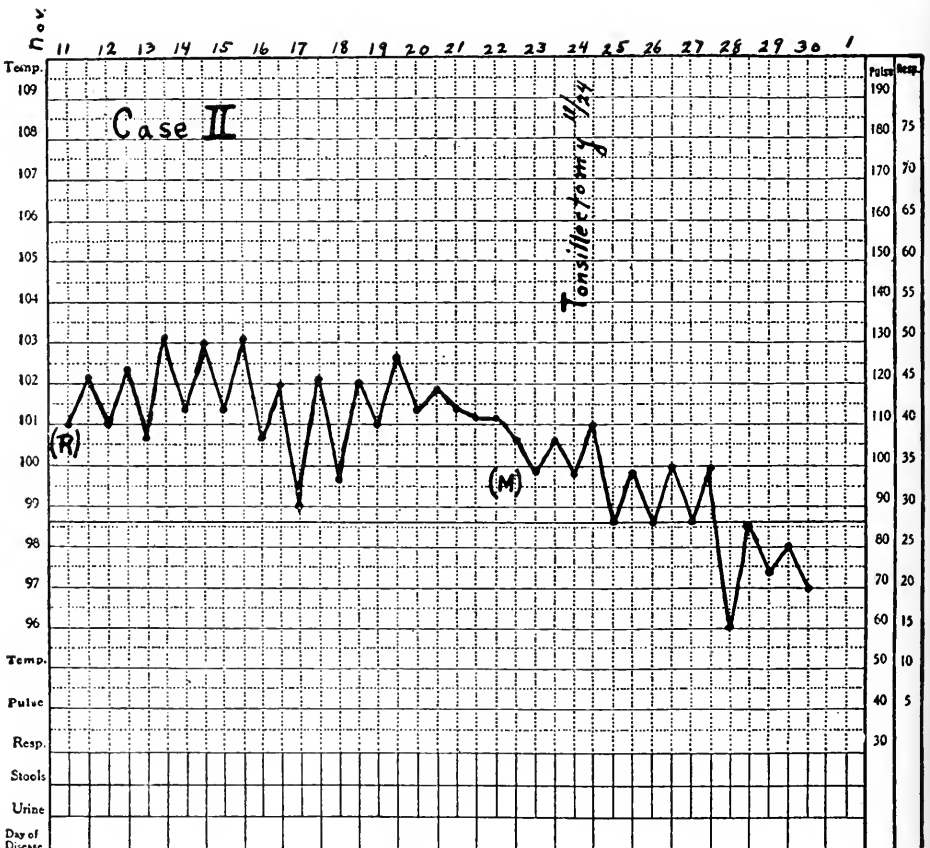


CHART I.



rhea alveolaris is present. There is a general reddening of the mucous membrane of the throat. Over the left tonsil which is large and ragged, there is a thin, grayish exudate. The right tonsil is also red and enlarged with a protuberance having a crater in the center. The pharynx is reddened and a small amount of purulent secretion is present. The cervical glands are not swollen.

*Heart.*—The point of maximal impulse is 9.5 cm. to the left of the median line in the 4th interspace. Relative dullness extends 3 cm. to the right in the 4th, and 12 to the left in the 4th. A blowing systolic murmur, which almost replaces the first sound, is audible at the apex and is transmitted to the axilla. The pulmonic second sound is accentuated. The blood pressure is 120 systolic, 80 diastolic.

There is swelling of each knee with increase in local temperature. The left knee is extremely tender to the touch. There is tenderness in the left hip.

The urine is negative except for the presence of a few finely granular casts. The throat culture is negative for diphtheria bacilli. The leucocytes number 16,500 of which 78% are polynuclear neutrophils. A blood culture on November 14th showed streptococcus viridans in pure culture.

Local treatment improved the condition of the throat very much by November 14th. The joint symptoms responded quickly to salicylates. The temperature nevertheless remained elevated, being between 101 and 102.5 and the leucocytosis persisted.

Tonsillectomy was performed November 24th. The temperature reached normal for the first time on the following day, and after the third day it remained normal (see Chart I).

The patient was discharged December 2nd, 1915, feeling well. In July, 1916, the patient was seen at his stall in the market and says he has remained in good health.

### CASE III.

*H. H.*, hospital No. 6619, a colored male, single, aged 48, a laborer, was admitted to the North Medical Service of the Cincinnati General Hospital, December 14th, 1915, complaining of pain in both legs and arms and of headache.

The family history is unimportant.

*Past History.*—For twelve years the patient has had frequent attacks of sore throat. He had acute arthritis 18 years ago and at this time was in bed for two months. Thirteen years ago the patient had a chancre. He admitted having had two or three attacks of gonorrhea; the last one was fifteen years ago. He drinks alcohol to excess.

*Present Illness.*—(Unfortunately the notes of the present illness are lost.)

*Physical Examination.*—The patient is a well nourished man. The teeth are in poor condition. There is marked pyorrhea. The soft palate and uvula are moderately injected. The anterior pillars are much reddened. The tonsils are enlarged and hyperemic. The pharynx is injected. The patient complains of difficulty in swallowing. The upper cervical glands are palpable.

The heart is normal.

There is pain in both knees and elbows on motion.

The urine is negative. The Wassermann is strongly positive. On December 21st the leucocytes numbered 8,800, of which 77% were neutrophils.

The temperature has been between 100 and 102.

Tonsillectomy was performed December 22nd, and deeply imbedded tonsils were removed. Within two days after the operation the temperature became lower and did not rise above 99.6. January 7th, the joint symptoms had disappeared and the patient was doing light work in the ward.

A blood culture made at the height of the disease was sterile. Treatment with mercury and iodide was begun December 16th. The patient was discharged February 16th much improved.

### CASE IV.

*F. E.*, hospital No. 6889, a white male, single, aged 27, a baker, was admitted to the North Medical Service of the Cincinnati General Hospital, December 26th, 1916, complaining of "rheumatism."

The family history is unimportant.

*Past History.*—The patient has had seven attacks of acute rheumatic fever. The first attack was fifteen years ago and the patient was in bed six months; the second attack

was in 1901, when he was in bed nine months; the third attack in 1902, in bed four months; the fourth attack in 1905, in bed five months; the fifth attack in 1907, in bed three and one-half months; the sixth attack in 1910, in bed two months. The total time in bed in the six previous attacks was twenty-nine months. The patient says he has been subject to mild attacks of tonsillitis, but he thinks they have had no relation to the rheumatic attacks.

*Present Illness.*—The present illness began December 17th, 1915, with pain in the great toe of the left foot. The following day he complained of pain and swelling in both knees and was unable to walk. The next day he suffered with pain in the back and now complains of pain in the right shoulder, wrist and knuckles and in the right knee.

*Physical Examination.*—The patient is a well nourished man of moderate size. Pyorrhea alveolaris is present. The soft palate, uvula and tonsils are moderately injected. The cervical glands are palpable.

*Heart.*—The cardiac dullness extends 11.5 cm. to the left in the 5th space, 4 to the right in the 4th space. A systolic murmur replaces the first sound at the apex and is transmitted to the axilla. The pulmonic second is accentuated.

The affected joints are red, swollen and very painful on passive motion.

The temperature is between 101 and 103. There are 18,800 leucocytes, with 85% of polynuclear neutrophils. Two blood cultures on December 26th and January 2nd were sterile. Gonococcus fixation test is negative. Wassermann test is negative. Urine is normal.

## CINCINNATI GENERAL HOSPITAL

NO. 6889 F.E. ADMITTED Dec. 26, 1915 WARD BT

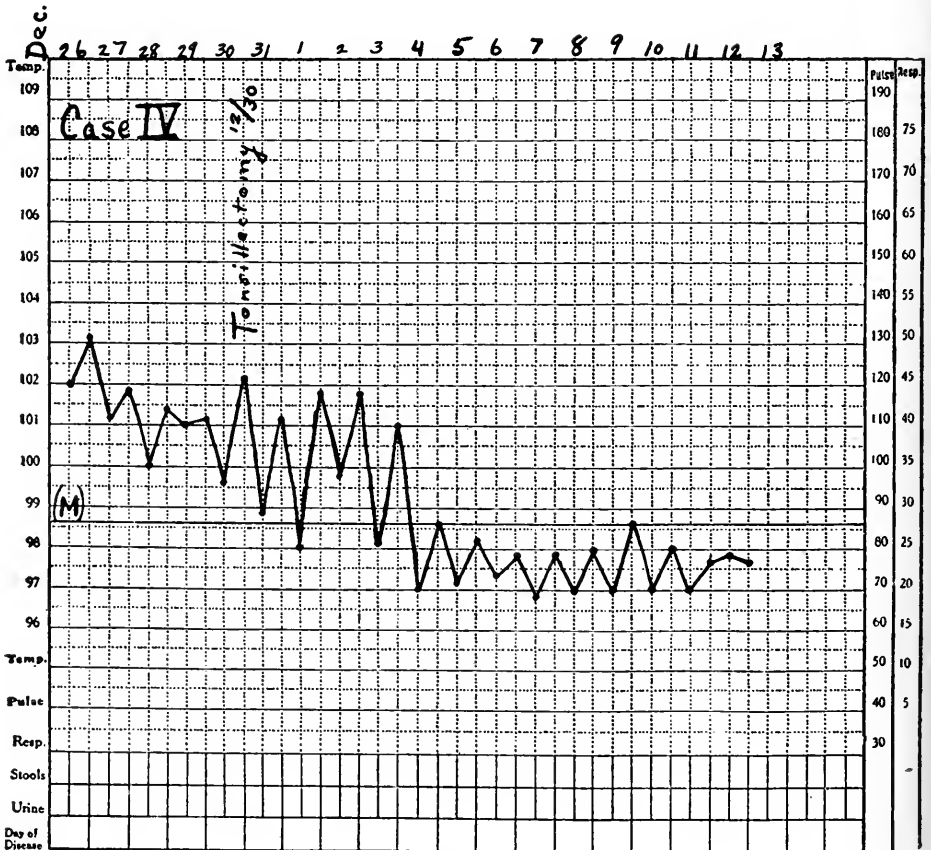


CHART II.

Tonsillectomy was performed December 30th. The temperature reached normal the fifth day after operation and remained so (see Chart II). The pain in the joints was much less marked by January 5th and on January 25th the patient was discharged, markedly improved.

X-ray plates of the teeth showed no abnormalities.

#### CASE V.

S. N., hospital No. A-5976, a white woman, single, aged 20, a shoe worker, was admitted to the North Medical Service of the Cincinnati General Hospital, September 5th, 1916, complaining of pain in the knee and finger.

The family history is unimportant.

*Past History.*—The patient has had frequent attacks of tonsillitis, and in the last four winters she has had an annual attack of quinsy and has been ill about two weeks with it as a rule. She has never had rheumatism before the present illness. She complains of slight dyspnea on exertion and occasional palpitation.

*Present Illness.*—On the night of August 31st, the patient complained of headache and soreness in the knee. She went to work the following day, but on September 2nd her physician sent her to bed. There have been no sweats. There have been soreness and stiffness in the knee and pain in the third finger of the left hand. There has been some nausea and vomiting.

*Physical Examination.*—The patient is a well developed, well nourished, rather anemic young woman. She is a mouth breather. The lips are thickened, the upper incisors prominent. The tonsils are hypertrophied, and the tonsils, pillars and uvula are reddened. There are no palpable glands in the neck.

The heart is somewhat enlarged, measuring 3.5 cm. to the right and 10.5 to the left. The point of maximal impulse is 8.7 to the left in the 5th space. The pulmonic second is accentuated, but no murmur has been heard over the heart. Blood pressure is 120 systolic, 60 diastolic.

The right knee and ankle are slightly swollen and the skin is warmer than on the left side; there is exquisite tenderness. There is also tenderness of the left third finger.

The urine is normal. The leucocytes are 20,000. Wassermann test is negative, as is also the gonococcus fixation test.

The Dental Service reported the teeth in good condition.

Tonsillectomy was performed September 9th. The patient had previously been given salicylates and alkaline drinks. Mouth temperature has not been above 99.6, pulse 100, respirations 26. Four days following tonsillectomy the temperature, pulse and respirations were normal.

The patient was sent to a convalescent home, September 16th, much improved.

#### CASE VI.

H. S., hospital No. A-788, a white male, single, aged 34, a machinist, was admitted to the North Medical Service of the Cincinnati General Hospital, February 2nd, 1916, complaining of "rheumatism from the hips down and inability to walk."

The family history is without interest.

*Past History.*—The patient says he has had sore throat every year since he was eighteen years old. The last three or four years he has had quinsy each year, with spontaneous rupture and expectoration of pus and blood. His first attack of acute arthritis was twelve years ago. Both ankles and knees were involved. The duration of this illness was twelve days.

*Present Illness.*—On January 18th, 1916, the patient had an attack of sore throat. There was great swelling of the tonsils, with rupture and escape of blood and pus, from the left side of the throat, the patient thinks. On January 24th, five days after the onset, the patient first noticed pain in his right ankle and foot. There has been successive involvement of the other ankle, both knees and the right hip. He has had no chills or sweats. He thinks he has had slight fever. The joints have been painful.

*Physical Examination.*—The patient is a well developed man. There are one or two infected snags, and there is moderate pyorrhea. The tonsils are moderately hypertrophied,

and the mucous membranes of the throat are generally injected. The lymphatic glands of the neck are generally enlarged. A consultation from the Throat Service was asked for and an infection of the left antrum of Highmore was detected and drained.

The heart is normal.

There is tenderness in the left hip, both knees and ankles, with redness and swelling of the joints.

The urine is normal. Leucocytes are 17,500, with 82.5% neutrophiles. Blood culture is sterile. Wassermann test is negative.

The temperature rose to 102 the first two days, then became normal.

The tonsils were removed December 10th. The pyorrhea has been treated by the Dental Service.

The patient was discharged well on March 21st. He remained in the hospital some weeks after the joint symptoms cleared up at the request of the Dental Service.

#### CASE VII.

*Miss B.*, aged 20, a nurse in training, complained of sore throat on February 11th and was admitted to the infirmary. She had been feeling poorly for about two days.

On admission her temperature was 102, pulse 120, respirations 20. She had acute follicular tonsillitis, temperature falling by lysis. It became normal on February 20th. It remained normal for four days. On the 25th of February the temperature rose to 99.6; on the 26th to 101.6. At this time she complained of abdominal pain and there was found localized rigidity and tenderness over McBurney's point. An operation was performed at once, and an acutely inflamed appendix was removed. The leucocytes at this time were 22,000. The temperature remained elevated between 100.4 and 103 until March 1st. It then ranged between 98.6 and 100 for several days. On the 28th of February the patient complained of aching in her limbs. The following morning, February 29th, both knees, shoulders, and some of the small joints of the hands were greatly swollen, red and extremely painful. The patient was given salicylates. The joint pains were controlled fairly promptly and the temperature came down, but the soreness and stiffness still remained to a certain extent. About March 10th, cultures were made from the right tonsillar region by Dr. E. C. Rosenow. Inoculation of two rabbits with this material produced an acute polyarthritis with multiple miliary abscesses in the myocardium. A staphylococcus was recovered from the tissues. The temperature began to rise on March 8th, and by March 13th it reached 102, pulse 100. Tonsillectomy, under local anesthesia, was performed on this date. The temperature rose to 103 the following day, and then for four days reached 102, gradually falling to a maximum of 99 on March 24th. Following the tonsillectomy the joint pains became worse for three days. About four days subsequent to tonsillectomy a mitral systolic murmur was first noted, and in spite of a return of the temperature to normal (though elevations to 99.4 or 99.6 occurred at intervals thereafter), the pulse rate remained high, varying for the most part between 90 and 110. After May 2nd, the temperature remained normal, the pulse was less frequent, and the patient was sent to her home for further convalescence.

#### CASE VIII.

*Miss X.*, aged 20, had an attack of acute tonsillitis early in March. About two weeks following this, tonsillectomy was performed under local anesthesia. The fourth or fifth day following tonsillectomy, the patient first noticed pain and swelling in the knees and ankles, and complained of weakness and prostration. She was admitted to the hospital on April 3rd, 1916. Temperature, 101, pulse 96, respirations 28. Previous to operation the heart had been examined and found normal. On admission there were found moderate dilatation of the heart, both to the right and left, with a systolic murmur at the apex and accentuation of the pulmonic second sound. The temperature became normal on April 9th and remained so. The pulse rate also dropped to between 70 and 80, on this date and remained normal. The dilatation of the heart became less, but the evidence of a mitral insufficiency persisted.

## CASE IX.

J. L., hospital No. A-777, was admitted to the North Medical Service of the Cincinnati General Hospital, February 2nd, 1916, complaining of vomiting of blood. The patient had several large hemorrhages, vomiting from one to two pints of blood at a time and the stools were tarry for several days. A diagnosis of gastric ulcer was made. He was given the Sippy treatment and made a complete recovery, the stools remaining free from blood after February 14th.

The patient was about to leave the ward when he developed an acute follicular tonsillitis on April 16th. April 21st the patient complained of pain in his left lower axilla, and a pleural friction rub was audible here. On the 25th blood was again found in the stools. On about April 24th the patient first complained of pain in his right knee which soon became swollen and hot and extremely painful on motion. The patient complained of some pain in others of the larger joints, but there was no definite redness or swelling. Blood cultures were sterile. The leucocytes were 28,000 on April 28th and there were 83 per cent of polymuclear neutrophils.

The patient ran a continued fever until May 3rd. The temperature then became intermittent, and after May 12th there were rises to between 99 and 100. During June there was only an occasional rise.

The arthritis in the right knee did not yield to salicylates and a subacute arthritis persisted. In August the patient complained of pain in his spine in the lumbar region and x-ray examination showed a beginning hypertrophic arthritis. Owing to the experience with Miss B. and Miss X., it was decided, after consultation with the Throat service, not to enucleate the tonsils during the febrile stage of the disease. The operation was performed, however, in August. Within a week thereafter, the knee began to improve and within a month there was practically no pain in it. The patient was also completely relieved of pain in the back within three to four weeks. He has worn a plaster jacket which may account for the entire relief of pain in his back.

## CASE X.

M. J., hospital No. A-5908, a colored widow, aged 45, was admitted to the North Medical Service of the Cincinnati General Hospital, September 2nd, 1916, complaining of "pain in the right side and back, misery in all the joints, fever and sweats."

The family history is negative.

*Past History.*—The patient had three miscarriages between the sixth and seventh months. She has had numerous attacks of sore throat since childhood. She never had acute arthritis.

*Present Illness.*—On August 17th, the patient complained of pain in the right side which radiated across the back. On August 22nd there was pain in the right shoulder and since then the pain has jumped from joint to joint. Fever and sweats began with the arthritis.

*Physical Examination.*—The patient is a short obese colored woman. There is marked pyorrhea alveolaris. The tonsils appear to be normal.

There is a systolic murmur at the apex. The aortic second sound is louder than the pulmonic second. The right ankle is swollen and hot. The knees are sensitive on pressure.

The urine is normal except for a few pus cells. Wassermann reaction is strongly positive. Gonococcus fixation test is negative. Blood culture is sterile.

The patient has an elevation of temperature to 100° F.

Tonsillectomy was advised by the Throat Service and performed on September 9th, 1916. The joint pains had been relieved by salicylates. The patient was entirely free from pain in her joints after September 15th. She was given mercury and iodide and was referred to the Dental Service for treatment of pyorrhea. The temperature continued to rise to 99.6 and it is probable that the alveolar infection accounted for this, as no other cause could be found. The patient was discharged September 26th improved.

## CASE XI.

L. S., hospital No. 6434, a white male, single, aged 56, a peddler, was admitted to the North Medical Service of the Cincinnati General Hospital, December 6th, 1915, complaining of "rheumatism."

*Family History.*—The patient's mother died of "cancer of the stomach."

*Past History.*—The patient has had rheumatism about ten years. He had four or five attacks, each of them associated with fever, he thinks. He frequently catches cold from exposure to the weather.

*Present Illness.*—The present illness began November 29th, 1915, with pain and swelling in the left wrist. Later the right hand and right ankle were involved, followed by pain and swelling in both knees and finally in the left ankle. The patient has been unable to walk. The present attack is similar to the previous ones, though less severe.

*Physical Examination.*—The teeth are in very bad condition. The gums are retracted. The tonsils show no particular change. The throat is generally reddened.

The heart is normal.

Both ankles are swollen. The right one is red and painful. There are no tophi. Blood cultures are sterile. Wassermann is negative. Gonococcus fixation test is negative. Leucocytes 9,700, 65.5% neutrophils.

On December 12th all of the carious and infected snags were removed.

The patient's temperature had been normal for three days and the joints were also painless. The patient was discharged on December 15th feeling well.

#### CASE XII.

R. K., hospital No. A-496, a white male, single, aged 52, a painter, was admitted to the North Medical Service of the Cincinnati General Hospital, January 21st, 1916, complaining of "rheumatism."

The family history is not of interest.

*Past History.*—The patient has had ten attacks of gonorrhea. For fifteen years he has averaged one attack a year of arthritis, involving the knees and ankles. The attacks have been of short duration, lasting three to seven days as a rule. The longest attack was of six weeks' duration and in this attack every large joint was involved. He was unable to work for three or four months after this attack. He drinks four to five glasses of beer and one glass of whisky daily. He gives no history of sore throat.

*Present Illness.*—The present illness began on January 18th, 1916, following four days' exposure to wet and cold. He complains of pain in both ankles and knees. He went to bed the day following the onset and came to the hospital two days later.

*Physical Examination.*—The patient is a well preserved man. The teeth are carious. There is marked pyorrhea alveolaris. The tonsils are not enlarged. Submental and posterior cervical glands are enlarged.

The heart is normal, except that the pulmonic second is louder than the aortic second.

There is swelling, redness and tenderness of the left knee and also tenderness and slight swelling of the left ankle.

The urine is normal. There are 7,800 leucocytes with no increase in the neutrophils.

The temperature rose to 101 on admission, gradually falling to normal on the 5th day.

The teeth have been treated by the Dental Service. The joint symptoms have disappeared. The patient was discharged well on March 8th.

General conclusions may not be drawn from so small a series of cases. It is encouraging, however, to note that the fever subsided promptly after the operation in some of the patients. In Case II, *Streptococcus viridans* was isolated from the blood on two different occasions by Dr. Wherry, subsequent to the control of arthritis by salicylates, and fever and leucocytosis persisted. Within three or four days after tonsillectomy, the patient's temperature became normal and remained so. The patient is a market man, exposed to the weather, but he has remained well since his discharge from the hospital, about eleven months ago. This is our most encouraging case; for the evidence (excepting a bradycardia) pointed to a *Streptococcus viridans* endocarditis. Subsequent events practically disproved this and make it appear probable that microorganisms were gaining access to the circulation directly from the tonsils.

In conclusion, it seems to the writer advisable, in patients suffering with acute rheumatic fever, in whom evidence of tonsillar infection, acute or chronic, can be obtained, to remove the tonsils as soon as the joint pains can be controlled, provided the main conditions, as outlined in the body of the paper, can be fulfilled. Further experience alone can determine whether the so-called complications and recurrences of arthritis can be prevented in this manner.

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## BACTERIEMIAS IN THE AGONAL PERIOD\*

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THE presence of a terminal infection coincident with the lowered bodily resistance usually present for a variable period just preceding death has long been recognized, but much less is known of the relative frequency and type of the bacterial invasion. We are familiar with the fact that any condition which depresses or diminishes the physiologic activity and vitality of the body diminishes its ability to defend itself against bacterial invasion and so predisposes to infection. These changes are often so subtle that they escape detection and at times the vitality of the host is so lowered that no systemic reaction is manifested and the presence of a bacteriemia remains unsuspected. With these facts in mind the idea was suggested that routine blood cultures be taken in consecutive hospital cases immediately after death and irrespective of the cause of the fatal issue. It is to be remembered that the isolation of bacteria just after death gives no clue as to the time when the invasion took place. In some cases the bacteria found were an important factor in the disease, while in others they merely represented a late or agonal infection occurring at a time when the body defenses were at a low ebb.

The significance of terminal infections, particularly those which have occurred in the course of serious metabolic disturbances where no organic destruction of surface tissues has taken place, is difficult to estimate. It would appear that the bacterial invasion was spontaneous and arose in areas which naturally harbor these organisms. To say that these local tissues had suffered a lowering of vitality until their resistance was unable to withhold the migration of bacteria into the body, is only expressing in general terms our ignorance of the actual mode whereby mucosal surfaces serve as a protective barrier to bacterial invasion. The fact remains, however, that this is true, and, furthermore, that the conditions necessary for bacterial invasion do not require the death of the protective mucosal tissues. These conditions become particularly available when the general bodily state of metabolism is at a very low ebb. It was this state which we desired to study from the standpoint of the types of bacteria which would first take advantage of the lessened tissue resistance. The influence which these terminal infections have in hastening the death of the patient, we were unable to study closely; but we have no hesitation in saying that where an individual is harboring as virulent an organism in his blood stream as the streptococcus pyogenes, he is being seriously injured by it.

Great care was taken in the collection of the materials to exclude errors which might influence the interpretation of the results. The collecting outfit which we used consisted of tubes of plain serum broth, which was the culture medium used in all cases, sterile capillary pipettes, an alcohol lamp, scalpel, sterile

\*From the Magee Pathological Laboratories, Mercy Hospital, Pittsburgh, Pa.



swabs, and tincture of iodine. These were kept in readiness and always available. Upon the death of a patient, the laboratory was immediately notified and the culture taken. The median basilic or cephalic vein was selected as the site for vein puncture. The skin was slightly drawn to one side and an incision made beside the vein, deep enough so that upon retraction of the skin the vein would stand out prominently between the everted edges of the incision. Tincture of iodine was then applied to the surface of the vein by means of a sterile swab. The tip of the sterile sealed capillary pipette was then broken, passed through the alcohol flame, and inserted into the vein. In the majority of cases, the capillary attraction and blood pressure within the vein was sufficient to fill the pipette with one or more c.c. of blood. The culture medium was immediately inoculated, and upon returning to the laboratory, placed in the incubator. The cultures were carefully watched for the appearance of growth and were without exception transferred to blood agar within twenty-four or thirty-six hours. Where a growth was observed, the organisms were plated, then picked for pure cultures, and subsequently transferred to their respective "sets." From the foregoing, it can be seen that the technic, while exceedingly simple, offers the least possible chance for contamination, inasmuch as the site of puncture does not come in contact with anything save the sterile swab saturated with iodine and the sterile capillary pipette. In only a few cases was any difficulty experienced in the removal of blood, and in these instances, it was due to the anemic state of the patient or to the small caliber of the blood vessels in children. The blood cultures were always taken within ten minutes following death and before the body had been in any way handled. This is a most important consideration; for even with the lapse of a very brief period after death, the manipulation of the body, such as occurs during its transference to the morgue, may cause the distribution of bacteria to sites other than those present during life. In all cases the cultural results here reported were obtained from materials collected from the body as it lay in bed.

From the following table it will be seen that out of the one hundred and nineteen cultures taken, forty-two were positive, and seventy-seven sterile. Furthermore, it will be noted that the organisms isolated belong to the types which are not uncommonly found in human infections of various tissues. While the number of positive findings may seem small, two factors should be considered: (1) the time postmortem, and (2) the type of case investigated. The time postmortem is important inasmuch as the possibility of a postmortem invasion of blood stream has been practically excluded. Postmortem invasion, however, is a very variable factor, as has been demonstrated by many investigators, and the possibility of such an occurrence is not to be overlooked. The type of case is also important, since in this series, consecutive cases were cultured irrespective of the cause of death. Then, too, the number of sterile cultures would indicate that the technic employed was a dependable one, since the ordinary contaminations were not present. The frequent sterility of postmortem blood cultures has also been the observation of other investigators. Strauch, in his study of two thousand postmortem blood cultures, found that in about one-half, or nine hun-

dred and ninety-eight times, the cultures were sterile. These were taken from the heart's blood at autopsy, and on an average of fifteen to sixteen hours after death.

TABLE I.

NO.	BACT. NO.	HOSP. NO.	SEX.	AGE.	CLINICAL DIAGNOSIS.	BACTERIOLOGICAL RESULTS AT TIME OF DEATH.
1.	939	1676	F.	37	Fibromyomata of uterus	No growth
2.	943	1820	M.	7	Ac. append. and peritonitis	No growth
3.	950	1936	F.	37	Puerperal septicemia	B. acidilactici
4.	951	1748	M.	66	Pneumonia—Cardiac dilatation	No growth
5.	968	1686	F.	48	Cholecystitis—Cholelithiasis	No growth
6.	972	1995	M.	39	Perforated gastric ulcer	Strept. salivarius
7.	999	2051	M.	69	Apoplexy—Chr. int. nephritis	No growth
8.	1013	2229	F.	24	Died during labor	No growth
9.	1017	2166	F.	28	Puerperal septicemia	No growth
10.	1021	2139	M.	16	Pneumococcic meningitis	Pneumococci
11.	1036	1996	M.	39	Sarcoma of lymph glands	Unidentified Gram-neg. bacillus
12.	1045	2019	M.	58	Carcinoma of prostate	No growth
13.	1065	2307	F.	35	Tubo-ovarian abscess	Staph. pyogenes aureus —Staph. albus
14.	1066	1804	M.	25	Lymphosarcomatosis	No growth
15.	1089	2131	M.	38	Ac. card. dilatation—Cirrhosis of liver	No growth
16.	1090	2295	M.	37	Chr. endocarditis—Coronary embolism	Strept. pyogenes— Staph. pyogenes aureus
17.	1098	2028	M.	36	Malaria—Sec. anemia	No growth
18.	1113	2349	M.	69	Cirrhosis of liver	No growth
19.	1117	2500	M.	44	Lobar pneumonia	No growth
20.	1121	2484	M.	24	Chronic nephritis	No growth
21.	1158	2541	M.	30	Chr. int. nephritis	No growth
22.	1175	2567	M.	21	Rupture of small bowel	No growth
23.	1193	2864	M.	65	Chr. endocarditis	No growth
24.	1229	3050	M.	31	Bronchopneumonia	Staph. pyogenes aureus
25.	1247	3045	M.	25	Typhoid fever	B. typhosus
26.	1270	2921	M.	49	Miliary tuberculosis	No growth
27.	1269	3109	M.	10	Supp. otitis media and meningitis	No growth
28.	1281	3135	F.	42	Pyosalpinx—Peritonitis	No growth
29.	1282	2968	M.		Diffuse peritonitis	Strept. viridans—Micrococcus?
30.	1332	2963	M.	36	Myelogenous leukemia	Strept. pyogenes
31.	1328	3342	M.	16	Hydrophobia	No growth
32.	1333	3381	M.	40	Lobar pneumonia	No growth
33.	1348	3391	M.	33	Delirium tremens	No growth
34.	1537	3395	M.	52	Ac. miliary tbc.	Strept. mitis—B. acidilactici
35.	1371	3320	M.	53	Chr. myocarditis.	No growth
36.	1373	3479	F.	7 mos.	Intestinal obstruction	No growth
37.	1390	3420	M.	33	Pneumonia and tuberculosis	Pneumococcus
38.	1402	3374	F.	36	Hyperthyroidism—Thyroidectomy	No growth
39.	1416	3551	M.	66	Lobar pneumonia	Strept. salivarius
40.	4	3753	F.	45	Hypernephroma of ureter	No growth
41.	27	3874	M.	32	Fracture pelvis—Traumatic ileus	No growth

TABLE I.

NO.	BACT. NO.	HOSP. NO.	SEX.	AGE.	CLINICAL DIAGNOSIS.	BACTERIOLOGICAL RESULTS AT TIME OF DEATH.
42.	28	3750	F.	42	Fibroid uterus — Cholelithiasis	No growth
43.	45	3146	F.	57	Pernicious anemia	Strept. anginosus
44.	47	3931	M.	54	Lobar pneumonia	Strept. salivarius— Strept. mucosus
45.	68	3940	M.	33	Lobar pneumonia	Pneumococcus
46.	69	3856	F.	47	Cholelithiasis	No growth
47.	70	3713	F.	18	Chr. arthritis—Ac. nephritis	No growth
48.	73	3956	M.	64	Lobar pneumonia	Pneumococcus — Staph. albus
49.	98	3897	M.	60	Ac. retention—Stricture of urethra	No growth
50.	102	4091	F.	84	Cerebral hemorrhage	No growth
51.	103	4092	M.	5	Hernia	No growth
52.	116	4207	M.		Lobar pneumonia	Pneumococcus
53.	127	3672	M.	47	Nephritis and pneumonia	Pneumococcus
54.	128	4213	F.	76	Strangulated hernia—Peritonitis	Strept. mitis—B. pseudo-diphtheria
55.	149	3717	M.	40	Chr. interstitial nephritis	No growth
56.	158	2311	F.	47	Pyosalpinx and pelvic abscess	No growth
57.	181	4485	M.	70	Lobar pneumonia	Pneumococcus
58.	184	4535	M.	55	Lobar pneumonia	Pneumococcus
59.	196	4405	F.	63	Complete prolapse of uterus	No growth
60.	213	4261	F.	51	Systocele—Rectocele	No growth
61.	214	4688	M.	25	Pulmonary tuberculosis	No growth
62.	216	4617	M.	34	Cerebrospinal lues	No growth
63.	231	4750	M.	35	Tumor of base of tongue	No growth
64.	257	4836	M.	43	General peritonitis	Strept. pyogenes
65.	258	4762	F.	29	Puerperal septicemia	Strept. pyogenes
66.	285	4885	M.	20	Septicemia	Strept. infrequens
67.	300	5086	M.	6	General peritonitis	No growth
68.	303	5032	M.	44	Lobar pneumonia	No growth
69.	306	5072	M.	53	Lobar pneumonia	No growth
70.	312	4293	M.	24	Broken back	No growth
71.	340	4866	M.	46	Appendiceal abscess — General peritonitis	Strept. pyogenes
72.	347	5315	M.	35	Lobar pneumonia	No growth
73.	368	5390	F.	2	Fecal impaction	Staph. pyogenes aureus
74.	369	5288	M.	41	Lobar pneumonia	Pneumococcus — Strept. pyogenes
75.	392	5253	M.	26	Pneumonia	Pneumococcus — Strept. pyogenes
76.	423	5491	M.	32	Cholecystitis — Appendicitis	Strept. pyogenes
77.	427	5493	M.	67	Hypertrophy of prostate	No growth
78.	448	5304	M.	72	Papilloma of bladder	Strept. equi
79.	454	5531	M.	67	Carcinoma of stomach	Strept. pyogenes
80.	459	4168	M.	44	Amebic dysentery	Strept. mitis
81.	470	5674	M.		Lobar pneumonia	Pneumococcus
82.	481	5702	M.	30	Lobar pneumonia — Pyo-pneumothorax	No growth
83.	482	5268	M.	5	Hemolytic anemia	No growth
84.	483	5550	M.	57	Septicemia following infected thumb	Staph. pyogenes aureus
85.	489	5910	F.	19	Puerperal septicemia	Strept. pyogenes
86.	498	5300	M.	48	Lymphosarcoma — Abscess of lung	Strept. hemolyticus Strept. viridans
87.	500	5946	M.	54	Fatty degeneration of heart	No growth

TABLE I.

NO.	BACT. NO.	HOSP. NO.	SEX.	AGE.	CLINICAL DIAGNOSIS.	BACTERIOLOGICAL RESULTS AT TIME OF DEATH.
88.	508	5947	M.	36	Chronic nephritis	No growth
89.	530	6094	M.	45	Carcinoma of stomach	No growth
90.	550	6289	M.	60	Pneumonia	Pneumococcus
91.	552	5766	F.	38	Heart disease	No growth
92.	562	6339	M.	19	Ruptured liver	No growth
93.	573	6363	M.	29	Acute alcoholism	No growth
94.	576	6283	M.	53	Diabetic gangrene of foot	No growth
95.	589	6257	F.	46	Dermoid cyst of ovary	No growth
96.	607	6441	M.	61	Aneurysm of aorta	No growth
97.	608	6156	M.	33	Alcoholism—convulsions	No growth
98.	666	6195	M.	40	Amebic dysentery	No growth
99.	669	6121	F.	67	Chronic pancreatitis	Staph. pyogenes aureus
100.	671	277	M.	72	Heart, kidney, and vascular disease	No growth
101.	690	6199	M.	44	Brain tumor	Pneumococcus
102.	709	511	M.	47	Pneumonia?	No growth
103.	714	2214	M.	21	Fracture 12th dorsal vertebra	No growth
104.	736	489	M.	26	Peritonitis—Ventral hernia	No growth
105.	737	602	F.	48	Intestinal obstruction	No growth
106.	742	205	F.	35	Tuberculosis of spine	No growth
107.	743	189	F.	58	Acute suppurative cellulitis of hand	Strept. salivarius— Staph. albus
108.	755	481	M.	36	Multiple burns	No growth
109.	762	6206	F.	39	Peritonitis following appendectomy	No growth
110.	763	194	M.	49	Carcinoma ampulla of Vater	No growth
111.	765	6540	M.	37	Abscess of liver	No growth
112.	770	639	F.	5	Meningitis	No growth
113.	772	658	F.	13	Peritonitis following appendectomy	No growth
114.	773	887	M.		Dislocation of hip	No growth
115.	774	905	M.	40	Pulmonary tuberculosis	Pneumococcus
116.	782	865	F.	62	Acute dilatation of heart	No growth
117.	784	956	M.		Heat stroke—Alcoholism	No growth
118.	785	925	M.	4 mos.	Plastic operation—Harelip	No growth
119.	791	792	M.	45	Pneumonia?	No growth

From the table it will be noted that of the forty-two positive cultures, thirty-one showed the presence of one organism, and eleven showed two organisms.

The frequency of the various types of organisms was as follows: streptococcus hemolyticus alone nine times, in all thirteen times; streptococcus viridans alone four times, in all ten times; pneumococcus alone eleven times, in all fourteen times; staphylococcus pyogenes aureus alone four times, in all six times. Staphylococcus albus was not found alone, but with other organisms, three times; B. acidi lactici alone once, in all twice; B. typhosus and an unidentified Gram-negative bacillus once. From the results obtained, it will be seen that the streptococcus was the most frequent organism isolated and the pneumococcus next. This was also the observation of Strauch whose results were as follows: of the one thousand and two positive cultures, streptococci were found alone four hundred and sixty times, in all five hundred and forty-eight times; pneumococci alone one hundred and fifty-five times, in all one hundred and ninety-seven times;

colon bacilli alone one hundred and thirty-two times, in all one hundred and thirty-seven times; staphylococci alone ninety-five times, in all one hundred and thirty-eight times; paratyphoid bacilli alone fourteen times, in all sixteen times; pneumo-bacilli (Friedlander) alone ten times, in all fourteen times; bacilli emphysematous (*B. welchii*) alone twice. Of the two thousand cases examined, the blood, therefore, was positive in 50.1 per cent; and in eight hundred and eighty-one times there was one type of bacterium, that is, 87.9 per cent of all positive findings; mixed infections of two types occurred one hundred and fourteen times, that is, 11.4 per cent; and a mixed infection of three types occurred only seven times, or 0.4 per cent of all cases.

As we have observed, and this would undoubtedly be substantiated in a larger series of cases, the streptococcus is the most frequent terminal invader. The occurrence of streptococcus pyogenes in cases 74 and 75, with the pneumococcus, is extremely interesting, and further careful study, in fatal cases of pneumonia, might show that the presence of such a coincident infection is relatively frequent.

In the course of the compilation of this data it occurred to us that a comparison of the bacteriological findings of the blood before and after death would be particularly interesting, in view of the fact that similar results would confirm the provisional antemortem bacteriological diagnosis, while the finding of additional organisms, would more firmly establish the more or less theoretical supposition of "agonal" or terminal infection. In reviewing the bacteriological reports extending over the period of investigation it was found that only fourteen antemortem blood cultures had been requested upon the one hundred and nineteen cases here studied. This is strikingly significant when we consider that in this brief series forty-two positive cultures were found. It further emphasizes that either the bacteriemias existed when the clinical manifestations of them were apparently not of sufficient import to justify the requisition of a blood culture, or that the infection had occurred very late in the progress of the disease when its presence had no clinical bearing. Unquestionably, bacteriemias, and often-times fatal ones, do exist where they are unsuspected. This, I believe, would be found particularly true in surgical cases where there is often a post-operative rise in temperature and an absence of the other generally recognized signs of blood stream invasion. However, it is not our purpose to discuss those bacteriemias existing for a considerable time before death.

We have long been familiar with the fact that overwork, previous infection, malnutrition, diet, intoxication, exposure, and trauma frequently lower the bodily resistance and predispose to infection. Why, then, should we not assume that morbid conditions in general, particularly immediately before death and when the bodily vitality is at its lowest ebb, offer the most favorable opportunity for a general bacterial invasion? This supposition, in order to be conclusive, must of course be proved by a greater number of cases than have here been presented, but it is earnestly hoped that the work may be continued. The securing of frequent antemortem cultures would be the most logical way in which to check the postmortem results.

The following table illustrates our results before and after death:

TABLE II.

NO.	BACT. NO.	HOSP. NO.	SEX.	AGE.	CLINICAL DIAGNOSIS.	A. M. DATE.	RESULT.	P. M. DATE.	RESULT.
1.	1008 1017	2166	F.		Puerperal septicemia	Oct. 5-15	Strept. pyogenes	Oct. 7-15	No growth
2.	1057 1090	2295	M.	37	Chr. endocarditis—Coronary embolism	Oct. 13-15	Staph. pyogenes aureus	Oct. 17-15	Strept. pyogenes Staph. pyogenes aureus No growth
3.	1209 1270	2921	M.	49	Miliary tuberculosis	Nov. 17-15	No growth	Nov. 28-15	No growth
4.	1227 1229	3050	M.	31	Bronchopneumonia	Nov. 20-15	No growth	Nov. 21-15	Staph. pyogenes aureus
5.	900 1138 1282	2968	M.	31	Diffuse peritonitis—Acute gangrenous appendicitis	Sept. 10-15	B. lactis aerogenes	Nov. 29-15	Strept. viridans micrococ- cus (?)
6.	1322 1328	3342	M.	16	Hydrophobia	Oct. 31-15 Dec. 7-15	No growth No growth	Dec. 8-15	No growth
7.	275	4885	M.	20	Septic arthritis	Feb. 29-16	Strept. infrequens	Mar. 3-16	Streptococcus infrequens
8.	316 333 340	4866	M.	46	Appendiceal abscess	Mar. 11-16	No growth		
9.	335 438 482	5268	M.	5	Hemolytic anemia (Aplastic)	Mar. 15-16 Mar. 18-16 Apr. 11-16	No growth No growth No growth	Mar. 18-16	Strept. pyogenes
10.	377 392	5253	M.	36	Lobar pneumonia	Mar. 29-16	No growth	Apr. 22-16	No growth
11.	456 483	5550	M.	57	Septicemia (infected thumb)	Apr. 16-16	Staph. pyogenes aureus	Apr. 1-16	Pneumococcus Strept. pyogenes Staph. pyogenes aureus
12.	488 489	5910	F.	19	Puerperal septicemia	Apr. 25-16 (4 hrs. A.M.)	B. acid lactici	Apr. 25-16	Strept. pyogenes
13.	625 643 651 765	6540	M.	37	Abscess of liver	June 1-16 June 7-16 June 9-16	No growth No growth No growth	July 3-16	No growth
14.	739 772	658	M.	13	Peritonitis following appen- dectomy	July 3-16	No growth	July 12-16	No growth

TABLE III.

NO.	BACT. NO.	HOSP. NO.	BACTERIOLOGICAL RESULT (IMMED. P. M.)	AUTOPSY NO.	TIME OF AUTOPSY	PATHOLOGICAL DIAGNOSIS.	BACTERIOLOGICAL DIAGNOSIS.
1.	950	1936	B. acidii lactici	A-34-15	2 hrs.	Rupture of uterus and peritonitis Lymphosarcomatosis Heart disease and coronary embolism	B. acidii lactici
2.	1066	1804	No growth	A-38-15	10 hrs.		No growth
3.	1090	2295	Strept. pyogenes Staph. pyogenes aureus	A-40-15	12 hrs.		Strept. pyogenes Staph. pyogenes aureus
4.	1123	2484	No growth	A-42-15	4 hrs.	Acute Bright's disease Lobar pneumonia	B. acidii lactici
5.	1229	3050	Staph. pyogenes aureus	A-47-15	6 hrs.		Staph. pyogenes aureus
6.	1247	3045	B. typhosus	A-48-15	1 hr.	Typhoid fever with hemorrhage Miliary tuberculosis Chloroma	B. typhosus
7.	1270	2921	No growth	A-50-15	11 hrs.		No growth
8.	1332	2963	Strept. pyogenes	A-52-15	4 hrs.		Strept. pyogenes
9.	1328	3342	No growth	A-53-15	2 hrs.	Hydrophobia and acute enteritis Hypostatic pneumonia	No growth
10.	1333	3381	No growth	A-55-15	2 hrs.		No growth
11.	1348	3391	No growth	A-56-15	6 hrs.	Infected clavus of toe with septicemia Pernicious anemia with cholecystitis	No growth
12.	45	3146	Strept. anginosus	A-1-16	1 hr.		No growth
13.	224	4691	No growth	A-10-16	2½ hrs.	Heart, kidney, and arterial disease Sarcoma of tonsil	Strept. anginosus
14.	231	4750	No growth	A-11-16	12 hrs.		No growth
15.	340	4846	Strept. pyogenes	A-14-16	3 hrs.	Appendiceal abscess with gen. peritonitis Amebic dysentery with chronic paren- chymatous nephritis	No growth
16.	459	4138	Strept. mitis	A-19-16	10 hrs.		Strept. pyogenes Strept. mitis
17.	530	6094	No growth	A-23-16	12 hrs.	Cancer of stomach	B. lactis aerogenes Strept. faecalis
18.	671	277	No growth	A-26-16	3 hrs.	Heart, kidney, and arterial disease Tuberculosis of spine	B. lactis aerogenes
19.	742	205	No growth	A-29-16	16 hrs.		No growth No growth

It will be noted that in cases 2, 4, 5, 8 and 12, additional or different organisms were isolated immediately after death. Case 12, presented a very interesting finding, inasmuch as four hours before death *B. acidi lactici* was isolated, whereas immediately postmortem, *streptococcus pyogenes* was found. These cultures were both taken from the same median cephalic vein, four hours apart, and yet each yielded a pure culture. In case 1, *streptococcus pyogenes* was isolated in the antemortem culture but was not obtained postmortem. In numbers 3, 6, 7, 9, 11, 13 and 14, the same results were obtained both antemortem and postmortem.

The value of late postmortem bacteriological findings, as obtained at autopsy, and particularly in those cases autopsied some time after death, has long been a subject of discussion. It was, therefore, believed that a comparison of cultures obtained immediately postmortem with those obtained later at autopsy would be significant.

The comparison suggested will be noted in the following table. The number, while too few to be conclusive, at least points out the possibilities of future investigations along this line. The cases, nineteen in number, were autopsied one to sixteen hours after death. Of that number, sixteen showed the same results at autopsy, while three showed further bacterial invasion. The post-mortem bacterial invaders were *B. acidi lactici*, *B. lactis aerogenes*, and *streptococcus fecalis*.

#### CONCLUSIONS.

1. Routine blood cultures taken immediately after death reveal the presence of an unsuspected bacteriemia in about one-third of all fatal cases.
2. Streptococci are the most frequent terminal bacterial invaders of the blood stream. The pneumococcus can be isolated in practically all cases of lobar pneumonia dying before the tenth day of the disease.
3. Bacteriological findings at autopsy within a few hours after death, though fairly reliable in demonstrating the presence of organisms existing at the time of death, do not exclude the possibility of postmortem invasion.
4. The taking of frequent antemortem, immediate postmortem, and autopsy cultures is to be encouraged. Contamination<sup>1</sup> may be obviated by a simple technic.
5. In the absence of adequate autopsy material, the routine taking of immediate postmortem cultures will furnish valuable information as regards the essential and terminal bacteriemias.

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## THE DIAGNOSIS OF GASTRIC ULCER

BY JOHN W. SHUMAN, M.D., SIOUX CITY, IOWA.

WHEN a "stomach complainer" consults, we should take a careful and complete history of that patient's health and record all the facts. If in the history such subjective symptoms as hyperacidity (sour eructations), hunger-pain, one to two hours after eating, which is relieved by the taking of food, and hypersecretion are stated, an ulcer of the gastro-duodenal area is to be differentially diagnosed from chronic appendicitis, cholecystitis, pancreatitis and oft-times other diseases.

If a thorough, painstaking, head-to-foot physical examination is made on this ulcer suspect and there is an absence of any visceral pathology demonstrable to inspection, percussion, and palpation, excepting a hypersensitive area or two in the abdomen, one or two tests should be made to exclude active ulceration. A general physical examination is extremely necessary. The finding of pus pockets in tonsils, teeth or elsewhere in a positive ulcer case demands eradication as a rational therapeutic procedure.

For the finding for or against active ulceration nothing is easier than the use of the No. 14 braided-silk cord and lead ball test. I have been using this test for a little over five years, first reporting it three years ago.<sup>1</sup>

The finding of a blood stain on the cord denotes bleeding from a raw surface and gives in a measure its location. The clean cord finds against ulceration.

I have checked this test against every possible source of error and have found it correct. In all cases operated upon in which the cord test was "positive" the surgeon found an ulcer. In one case, the cord showed two blood stains; two separate and distinct ulcers were found at autopsy.

The use of the duodenal tube is quite necessary in many cases in which one is attempting differential gastric diagnostic work. By using it carefully and observing, one can tell when the bulb passes into the duodenum. If blood (bright red shreds) is aspirated prior to the bulb passing out of the pylorus, ulceration on the gastric side is evident; if after passing the pylorus blood is aspirated, an ulcer on the duodenal side of the pylorus is just as evident. By securing the duodenal secretions and examining one can also tell something of the biliary and pancreatic function.

For differential and confirmatory diagnosis the roentgen ray is indispensable. I do not believe any one test sufficient to establish a diagnosis. All methods of diagnostic technic should be used in a given case. When they are not used it is either because of laziness, inefficiency or uninterestedness. The more careful, thorough and conscientious our work, the fewer mistaken diagnoses will result.

In the roentgen ray examinations of the hollow viscera of the abdomen, I follow the plan of using two to three ounces of barium sulphate and twenty-four to thirty-two ounces of buttermilk for the opaque meal. Six ounces of

<sup>1</sup>St. Paul Med. Jour., April, 1914, later Jour. Lancet, Aug., 1916, and Kan. City Med. Herald.

barium sulphate and thirty-two ounces of "stale" buttermilk (98° F.) are used for the opaque enema. Examination is made with the roentgenoscope while the opaque matter is passing into the stomach or colon. If any abnormality is noted an "immediate" exposure is made for record. At six hours the stomach is again examined. Ofttimes several exposures are necessary before a correct interpretation can be attempted.

The roentgen ray findings upon which I place the highest value are, first, a diverticulum; second, a permanent incisura opposite the site of the ulcer; third, hyperperistalsis (duodenal ulcer); fourth, a large six-hour residue (pylorospasm); and fifth, a deformed cap or duodenum, which shows constantly in a series of plates (duodenal ulcer). Hyperperistalsis and a large six-hour residue are highly suggestive, and if in addition we have a permanent incisura opposite the site of the ulcer we can be certain of the presence of ulcer. A diverticulum is practically diagnostic of penetrating ulcer. If a deformed cap or duodenum is present I feel safe in diagnosing duodenal ulceration from the roentgenoscopic examination.

Here are two cases reported in full for comparative study in differential gastric diagnostic technic. Both complained of "stomach trouble," both were referred for "treatment of gastric ulcer." One had ulcer of the stomach, the other chronic appendicitis.

*Case 1.*—Mrs. S. Married, age 40. Was referred Feb. 5th, 1916, complaining, "stomach has been hurting a long time."

Family history, unimportant. Previous diseases: scarlet fever at 25; at 36 confined to bed for four weeks with "inflammation of the left lung." At 38 a part of the left nipple was excised; diagnosed "Paget's disease."

Present illness; began one and one-half years ago with bloating after eating; pain severe two to three hours after eating; vomiting relieved gastric hypertension. Weight then 125 pounds, now 109¾. At present, "aches through abdomen" and is stiff in limbs and back; stomach is sour; "baking soda relieves the stomach symptoms." Constipated. Menses very irregular.

Nocturia            1   2            Sleeps fair.

Day nocturia    4   5

Physical examination; T. 99.2, P. 119, R. 25. Hemanalysis; Hgb. 75% W.B.C.8,300. R.B.C.3,800,000. Wassermann negative. Urinalysis: straw, cloudy, acid, 1026, phosphates positive. Albumin, sugar and microscopic examination negative.

Mouth; severe pyorrhea of all but four lower front teeth. Tonsils submerged and diseased. Chest negative for active disease findings. Abdomen; a tender movable, walnut-sized mass just above and a little to the right of the navel (thought to be pylorus). Vaginal and rectal examination, negative. Extremities; second degree varicosities of left lower limb. Stool—occult blood positive (meat-free diet). Braided-silk cord test positive. Fig. 1 shows this cord with a two and one-half inch blood stain upon it, just a short way from the bile stain. roentgen ray examination showed a fish-hook stomach and a deformed cap which was constant through a series of (7) exposures. (See Fig. 2.)

Operation was advised on account of the duration and severity of symptoms

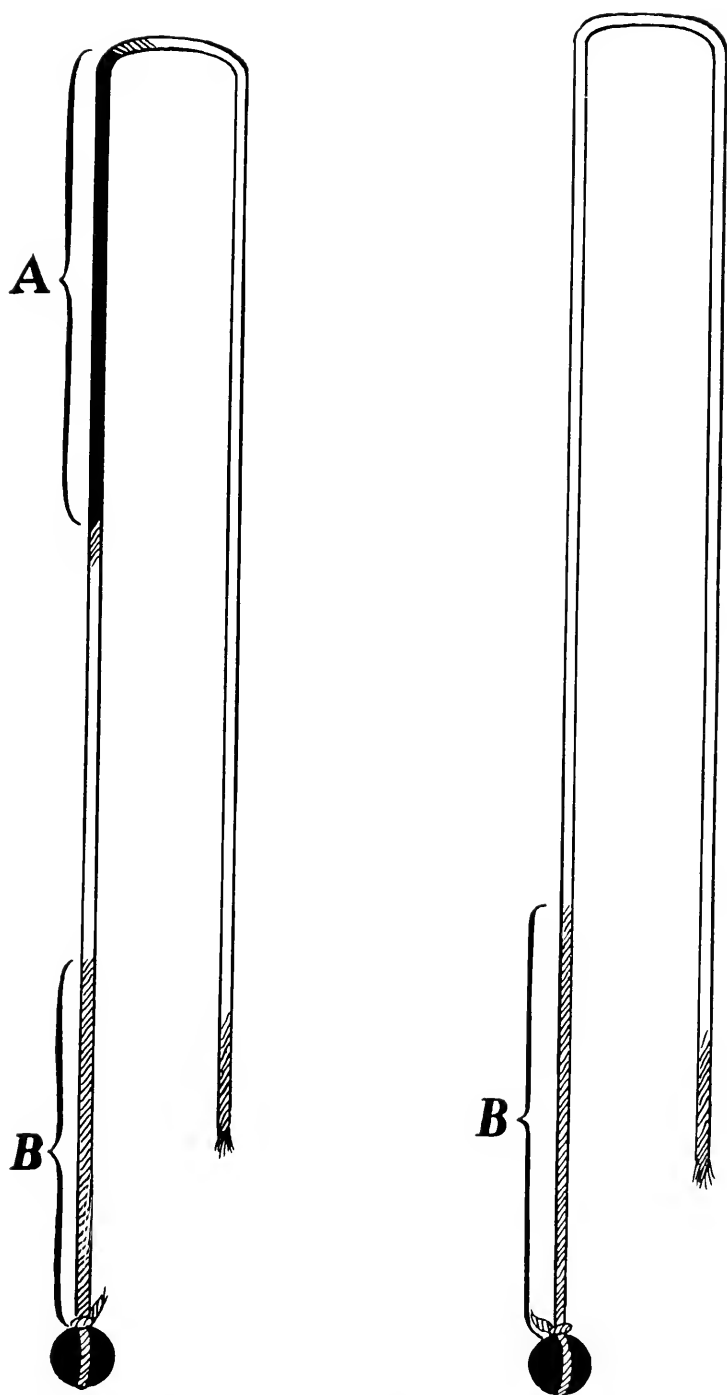


Fig. 1.—Actual size of cord and ball used in Case 1. The length of the string was 30 inches. *A* shows the two and one-half inch blood stain (dark red) and *B*, the bile (yellow) stain with a clear space intervening. Compare with the negative cord on the right of Case 2.



Fig. 2.—Case 1. Position prone; time, 30 minutes. Reading; enlarged, fish-hook shaped stomach. (A) Pylorus. (B) Deformed duodenal cap which was constant throughout examination. Compare with Fig. 3—a normal cap.



Fig. 3.—Case 2. Position prone; time, first meal 74 hours, second meal 10 min. Reading, normal stomach and cap. (A) Gastric spasm which did not remain constant during examination; diagnosed as reflex caused by (B) a clubbed appendix which did not empty for ten days when all other organs were clear of barium in forty-eight hours.

and the history of malignancy of the left breast, but the patient refused. Her diseased tonsils and teeth were removed. She was put to bed and the non-irritation treatment for pyloric ulcer administered. At the end of six weeks she had gained six pounds in weight and lost all feeling of discomfort and pain, the tender walnut-sized lump in the pyloric region had also disappeared. At the end of six months (Aug. 5th, 1916), she had gained ten pounds and was "feeling the best she had for three years." The time is too short to say more of the "cure."

*Case 2.*—Mr. B., single, age 23, clerk, was referred June 2nd, 1916, complaining of "stomachache." Family history; one brother dead at 31, "gall stones" (unoperated). Previous diseases; left sided pleurisy off and on for 2 to 3 years. One and one-half years ago operated upon and a pea-sized calculi was removed from the left ureter; since then no pleurisy. Present illness; began four weeks ago with almost constant nausea. No appetite for three or four days. Since then no trouble until four days ago when he suffered a similar attack. Soon after eating, his stomach hurts and continues to pain until the next meal. Has lost seven pounds in six weeks.

Physical examination; T.99. P.85. R.16. Hemanalysis; Hgb. 85%. W.B.C.-9,000. R.B.C. 4,500,000. Urine; straw, clear acid, 1020. Phosphates positive, albumin, sugar and microscopic tests negative. Stool; occult blood, negative. Head and chest negative. Upper abdomen negative. Tender McBurney point. Rectal and genitalia examination negative. Roentgen reading; a clubbed appendix causing a reflex spasm of the stomach. (See Fig. 3.) Operative findings confirmed this diagnosis as did the subsequent history, i. e., uneventful recovery and no gastric disturbance since.

Many diseases can simulate gastric disease until a thorough examination is made.

# LABORATORY METHODS

## THE DETECTION OF SEMEN

BY VICTOR C. VAUGHAN, M.D., ANN ARBOR, MICH.

IT is understood that in rape there may not be either immissio penis or emissio seminis though both may happen and either may occur without the other. In women not virgins defloration cannot occur because it has already happened, but in all cases the investigation should include a careful search for semen which may be deposited in the vagina, between the labia, on other parts of the person, on the clothing, on the bed, floor and ground. In case death has resulted the search for semen should not be confined to the locations mentioned but should extend to the uterus and Fallopian tubes. Unfortunately the polluted victim frequently washes herself and possibly her clothing before an examination can be made. In favorable cases spermatozoa may be found hours and days after the act. Mashka found spermatozoa in the vagina of a girl twelve hours after she was raped, and the same author quotes Downe who found spermatozoa in the vagina of a woman who had been in the hospital for some days, and Bayard, who was successful in his search three days after copulation. Bossi states that elements of the semen may be found in the vagina from twelve to seventeen days after coitus, while Hausmann was unable to find them after thirty-six hours. Haberda states that when the genitals are healthy the spermatozoa may, within a few hours, find their way into the Fallopian tubes. Experimentally this has been found to happen in some of the lower animals within two hours, and there seems to be no reason why it should not occur with equal promptitude in woman. When the secretions are healthy, living spermatozoa may be found a long time after the last coitus. Dührssen, in an operation for pyosalpinx, found motile spermatozoa in the less affected tube, although the woman had been in the hospital nine days and three and one-half weeks had passed since the last coitus. Spermatozoa may retain their vitality in the dead bodies of animals for some days and Ivanoff found that they may fecundate when taken from the vesicles twenty-four hours after death. In two cases in which death occurred during or immediately after coitus, Haberda found spermatozoa in the vagina, but after a most careful search failed to find any in the uterus or Fallopian tubes; however, this author states that Zweifel reports an instance in which death occurred during coitus and sixteen hours later motile spermatozoa were found in the Fallopian tubes, but these may have come from a previous copulation. It seems from these statements that the finding of spermatozoa in the uterus and tubes does not prove conclusively that coitus has immediately preceded death unless it can be shown that there had been no recent, but prior, indulgence in the sexual act.

The external genitals should be carefully examined for seminal stains. With only vulval penetration or even when the connection does not go so far

as this there may be *emissio seminis*. We must also recognize the fact that some of the old sexually exhausted men who commit rape on children select their victims because they are unable to copulate with adult women, and some of these blennorrhagic individuals have no spermatozoa in their semen. In such instances the proof that a stain is seminal cannot be positive and complete.

Some authorities have placed considerable stress upon the contour, gray coloration and odor of seminal stains especially when deposited upon white cloth, but it is sufficient to state that in none of these appearances is there anything sufficiently characteristic of seminal stains to enable the investigator to arrive at a positive conclusion, and the statement of the expert must be founded upon incontrovertible fact and not upon mere belief or suspicion. The only conclusive evidence that a stain is of seminal origin depends upon the finding of spermatozoa. Other morphological elements may or may not be present, but spermatozoa only characterize seminal fluid and unless their presence be demonstrated the evidence must be considered as negative. Epithelial cells, so-called seminal granules and spermatid crystals may or may not be present in seminal fluid and some of them certainly are found in stains from other sources. No one should attempt to decide concerning a suspected seminal stain unless he is thoroughly familiar with the appearance of spermatozoa in both fresh and dried specimens. In freshly ejaculated semen and in the mucus of the normal female genitals, they may retain their motility for a long time and in dried stains they may preserve their form and may be easily recognized under the microscope even after many years. If the stain be upon cloth, leather, wood or metal and if it be of sufficient thickness it is better to detach a bit of it from the object to which it is adherent and rub this up with a few drops of physiologic salt solution and examine it under the microscope. If the stain cannot be detached from the substance to which it adheres and which generally consists of some article of clothing, a small bit of the cloth carrying the stain should be cut out, placed in a watch glass, treated with a few drops of salt solution, covered so as to prevent evaporation and to protect it from dust and allowed to stand for two or more hours. Usually this results in the formation of a milky fluid, a drop of which may be subjected to direct microscopical examination. Another method is to place a bit of the cloth carrying the stain on an object glass, add a few drops of water or physiologic salt solution and with needles tear the fibres of the cloth apart; then, cover with a thin glass and examine with the microscope. When the stain is very old it is well to render the salt solution used for maceration feebly alkaline with ammonia. When a more active solvent is needed, as in the case of the presence of a large amount of dried mucus, a dilute solution of potassium hydroxid or a five per cent solution of acetic acid may be employed. Spermatozoa are fairly resistant to both acids and alkalis. Roussin recommends that the stain be soaked in a solution made up of 1 part of iodine, 4 parts of potassium iodid and 100 parts of water, and Unger advises that the spermatozoa be stained with a solution of methyl green (0.3 g. of methyl green to 100 of water, acidified with 6 drops of hydrochloric acid).

It is not safe to depend upon granules that appear to be the heads of



spermatozoa, because there are too many things that may be mistaken for these. More or less complete cells with both head and tail should be found before a positive conclusion is reached.

When the mucous secretion of the genitals is semi-fluid it is sufficient to take a drop of it and make with this a thin smear and examine directly with the microscope. When the mucus is dry a few drops of a salt solution may be added in order to dilute and dissolve it.

Florence has discovered a method of preparing from seminal stains crystals which he believes to be characteristic of spermatic fluid. The reagent employed consists of the following ingredients: Potassium iodid, 1.6 g.; iodine, 2.54 g., and water 30 g. This reagent should be cooled by surrounding it with ice water for some hours or by keeping it in an ice box for twenty-four hours before the test is made. The examination is carried out as follows: A bit of the suspected stain in a drop of the maceration obtained from it is placed on a glass slide, then a drop of the reagent is placed sufficiently near so that the two when covered with the thin glass gradually flow together. The observer looking through the microscope will see the crystals form as the two fluids come in contact. These crystals are brownish, rhombic prisms closely resembling those of hematin, although there may be many atypical forms. They are soluble in water, alcohol, acids and fixed alkalis. Individual crystals may be large enough to be seen by the unaided eye. They are not permanent, though according to Richter they may be preserved for a time, at least, by the addition of a few drops of a solution of iodic acid and enclosing in a cell of wax. According to Florence the formation of these crystals is characteristic not only of seminal stains, but of those from man, inasmuch as he finds that they are not found in the spermatic fluid of other animals. He believes that the presence of these crystals, even when spermatozoa cannot be detected is evidence that the stain is made by the spermatic fluid of man. This is important if true, but Richter holds that the crystals may be obtained from many substances, such as decomposing egg-white, egg-yolk, pus, vaginal and uterine mucus, the semen of the lower animals, certain vegetable substances and butter, and Gunrecht suggests, with great probability, that they are lecithin compounds and that when putrefaction advances far enough to break up the lecithin molecule even human semen does not give them. Haberda states that in some badly decomposed vaginal secretion taken from a corpse he found many well formed spermatozoa, but could not obtain the Florence crystals although his reagent gave good results with other seminal stains and with the semen of a man with azoospermia. This shows that the proposition of Binder that the Florence test be used as a preliminary one and that when it fails the search for spermatozoa need not be continued is not wise. Haberda, however, thinks that the test may be of service in picking out the one stain among many most likely to show spermatozoa. Dvornitschenko finds that the Florence test is not possessed of positive value, that there are substances that do not come from seminal fluid that yield the crystals and that when the reaction is negative spermatozoa may be found. He concludes that the substance in seminal fluid which takes part in the formation of the crystals is not a constant constituent of the secretion. In short, this

author agrees with many others in finding that Florence's reaction is not possessed of absolute value, and that it is of service only as a confirmatory or supplementary test in the identification and recognition of spermatozoa. It seems, therefore, that we must hold to the old view that the detection of spermatozoa is the only sure proof that a stain is made by semen and the finding of these cells needs no confirmatory or supplementary test.

Staining spermatozoa in order to bring them more distinctly to view has been recommended, and we have already referred to the methods of Roussin and Unger. Florence uses crocein, and Grigorgew recommends the following method: A bit of the cloth carrying the stain is placed in concentrated sulphuric acid and left until the fibre is quite well destroyed. This may require seventy-two hours, but as a rule the partly disintegrated fiber, after four, eight or twelve hours, still saturated with acid, is placed on a glass slide, covered and examined under a magnification of 600 diameters. The heads of the spermatozoa are oval or pear shaped and brown in color, and show a lighter point or vacuole. The tail is not colored so deeply. The method is not applicable to stains on brown or black cloth. It seems that some years ago Vogel recommended a somewhat similar method; the bit of cloth is moistened with water, placed on the glass slide, two drops of sulphuric acid and, after two minutes, the same amount of tincture of iodine is added, after which the examination is made. It is claimed that the sulphuric acid method is of special value when the stain is found upon a much soiled piece of cloth, and that everything save the spermatoc cells is destroyed by the strong acid.

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## TRYPSIN BROTH—AN IDEAL MEDIUM FOR MAKING BLOOD CULTURES

### A Preliminary Report\*

BY R. G. OWEN, M.D., F. A. MARTIN, M.D., AND W. G. PITTS,

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WHILE working on the treatment of suppurating wounds, Sir A. E. Wright observed that when the antitryptic activity of the serum was destroyed by the trypsin set free from disintegrating leucocytes, many organisms grew luxuriantly which were incapable of growth in the presence of serum rich in antitryptic power.

Emery further showed that by neutralizing the antitryptic action of serum with trypsin, the blood lost its coagulability, and with this also went a loss of opsonic, complementing, and bactericidal action.

Reasoning from this basis, Douglass and Colebrook decided that the addition of proper amounts of trypsin to beef-tea would furnish an ideal medium for blood cultures, and certain experiments which they made corroborated their views.

\*From the Bacteriological Department of the Detroit Clinical Laboratory.

We have been able to confirm their results in the main; and from our experimental and clinical results, can most strongly recommend "Trypsin Beef-Tea" as an ideal medium for making blood cultures.

We have tried various preparations of trypsin in different strengths, and have found the "Liquor Pancreatica" made by the Digestive Ferments Co., of Detroit, Michigan, to be the most satisfactory.

The Digestive Ferments Co. supply this preparation, sterilized by passage through a Berkefeld filter, in 10 c.c. ampules.

This amount should be added to 90 c.c. of sterile beef-tea and 5-10 c.c. of the mixture put in sterile test tubes. The medium must be handled aseptically, as heat destroys the action of the trypsin. When making cultures, add 1 c.c. of blood to each of 5 to 10 tubes of medium.

Trypsin beef-tea, we have found, keeps its strength for at least four weeks. Staphylococci, streptococci, pneumococci, and typhoid bacilli from the blood stream, all show a much earlier and more uniform growth in the trypsin medium than do cultures in plain or glucose beef-tea.

We would greatly appreciate any case reports dealing with the use of this medium in isolating organisms from the blood.

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## A STANDARD METHOD FOR MAKING UNIFORM COLLOIDAL GOLD SOLUTION

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BY WILLIAM K. TRIMBLE, M.D., KANSAS CITY, MO.

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**A**LL glassware should be rinsed successively with a small amount of freshly distilled water, neutral 95% alcohol, ether and finally heated in a hot air oven.

The water used should be freshly distilled in an all-glass still without rubber connections. Redistilling is not necessary.

The solutions of gold chloride, potassium carbonate and formaldehyde solution are prepared in the usual way and kept in a dark place in well fitting glass-stoppered bottles.

One should have a separate pipette for each solution used in the technic and these should not be used for other laboratory work.

Only chemically pure reagents should be used. In making the 1% formaldehyde solution treat the usual 40% as 100%.

*Technic.*—Distill 500 c.c. of water into one of the especially cleaned Erlenmeyer flasks and place on an iron tripod. Interpose between the flame and the flask a disc of copper about the thickness of a heavy blotting paper. The object of the copper disc is to permit a more uniform heating of the water and at the same time prevent overheating the solution at points in the bottom of the flask. Insert a clean thermometer into the water and when the temperature has reached 65° C. add at once 5 c.c. of the 2% potassium carbonate solution. Stir, and when the solution has reached 75° C., immediately add 5 c.c. of the 1% gold chloride

solution. Stir and bring the temperature of the solution to 90° C. Remove the flame and begin adding the 1% formaldehyde solution quite a few drops at a time and with constant stirring. It is important not to be hasty in adding the formaldehyde solution. When 3 to 3.5 c.c. have been added, allow considerable time before more is used, then only a few drops at a time. In a short while a perceptible change in the color of the solution will be seen, when no more of the formaldehyde solution should be added. Let the flask remain on the hot copper disc until the solution changes to a bright, clear, deep red color, when the flask is removed and allowed to cool. The color deepens slightly on cooling.

It is not necessary to reheat during or after the addition of the formaldehyde solution, so long as the whole remains at 90° C.

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## THE PRESERVATION OF ERYTHROCYTES FOR THE WASSERMANN REACTION\*

BY STANLEY P. REIMANN, M.D., CLEVELAND, OHIO.

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NUMEROUS methods have been devised for the preservation of erythrocytes for use in the Wassermann reaction and in other immunologic procedures. The time, trouble, and expense of the weekly or semi-weekly trip to the abattoir can be much reduced if a simple and satisfactory method can be utilized which will give results with preserved cells identically the same as results obtained with fresh cells. The present study compares results obtained with fresh cells and cells preserved by the formalin method and by the saccharose-gelatin method of Rous and Turner. For this purpose 200 tests were made with cells preserved by the Rous Turner method and 150 tests with both the Rous Turner and formalin methods. In all cases simultaneous tests were made with cells from 1 to 4 days old kept in the ice chest. Complement and hemolytic amboceptor were titrated separately against each sample of cells used and in the vast majority of cases, the results were identical; differences when found were small.

The formalinized cells were satisfactory for at least three weeks, several specimens for four weeks. The first cells preserved by the gelatin and saccharose solutions were stored in cotton-stoppered vials and hemolysis in sufficient amount to render the cells useless occurred in eighteen days. Those preserved subsequently were placed in sealed ampules and they were satisfactory for from 21 to 25 days.

The discrepancies in the final readings amounted to 6 per cent of the total number but no uniform variation was to be discerned. In some instances the preserved cells gave negative readings and the fresh cells positive readings, and in other instances the readings were reversed. In all these cases, the four antigens used,—two cholesterinized alcoholic extracts of human hearts, one cholesterinized alcoholic extract of ox hearts, and an alcoholic extract of syphilitic

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\*From the Departments of Pathology and of Dermatology, Western Reserve University.

liver, gave contradictory readings with preserved and with fresh cells. Reference to the clinical histories of many of the patients from whom these sera were obtained gave evidence of treated lues in various stages.

The method of preservation as worked out by Rous and Turner was carried out in the following manner: The blood of a sheep was run from the carotids directly into Locke's solution containing one per cent sodium citrate in the proportion of one part of blood to four parts of solution. The corpuscles were separated by rapid centrifugalization and carefully washed three times in Locke's solution containing 0.25 per cent gelatin. The cells were then placed in a layer not more than 2 mm. in depth and covered with saccharose-Locke solution to a depth of about 2 cm., the ampules were sealed and stored at a temperature of 5° C. and 6° C. Just prior to use the cells were washed once with .85 per cent saline solution to remove the saccharose solution, and proper dilution effected with saline. Strict asepsis was observed.

The cells for formalin preservation were prepared by allowing the sheep's blood to run directly into formalin solution in the proportion of 0.5 c.c. of 40 per cent formaldehyd solution to 400 c.c. of blood. Defibrination was accomplished by shaking with glass beads. The mixture was not disturbed until ready for use, when the cells were washed three times with 0.85 per cent saline solution in the usual way.

#### THE SOLUTIONS.

##### Locke-sodium citrate solution.—

Sodium citrate.....	10	grams
Sodium chlorid.....	9.2	"
Sodium bicarb.....	0.05	"
Potassium chlorid.....	0.1	"
Calcium chlorid.....	0.1	"
Aq. dest. q.s. ad.....	1000	c.c.

##### Locke-gelatin solution.—

Gelatin .....	2.5	grams
Sodium chlorid.....	9.2	"
Sodium bicarb.....	0.05	"
Potassium chlorid.....	0.1	"
Calcium chlorid.....	0.1	"
Aq. q.s. ad.....	1000	c.c.

The Locke and saccharose solutions are sterilized separately and used in the proportion of 2.8 c.c. of the saccharose solution and 7.5 c.c. of the Locke's solution.

##### Saccharose solution.—

Saccharose .....	103.0	grams
Aq. q.s. ad.....	1000	c.c.

##### Locke's solution.—

Sodium chlorid.....	9.2	grams
Sodium bicarb.....	0.05	"
Potassium chlorid.....	0.1	"
Calcium chlorid.....	0.1	"
Aq. q.s. ad.....	1000	c.c.

## CONCLUSION.

Under ordinary laboratory conditions sheep erythrocytes for use in the Wassermann reaction can be preserved satisfactorily from 3 to 4 weeks by the formalin method, and for from 21 to 25 days by the Rous-Turner method. The readings obtained differ from those obtained with fresh cells only in so far as some sera produce slightly different results when used with cells from the same specimen of sheep blood.

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## A SIMPLIFIED COMPLEMENT FIXATION TEST\*

BY NORMAN E. WILLIAMSON, M.D., STOCKTON, CALIF.

THE following method is a modification of Noguchi's test, but is much simpler and quicker.

Capillary pipettes are drawn out of small glass tubing. With a light touch of a file a mark is made at the point which 0.06 c.c. of mercury reaches from the end of the capillary tubing. The entire length of the pipette is 12 cm. Blood is drawn from the ear to the mark. This will contain approximately 0.02 c.c. of serum for each tube of the test as used in the Noguchi test, in addition to cells.

Place the small tubes in the rack used for complement fixation and add to the front tube 1.94 c.c. of citrated salt solution. The salt solution is the usual 0.9 per cent. The citrate is added in the proportion of 4 parts to 10,000. It is convenient to keep on hand salt solution containing 1 per cent citrate. Four c.c. of this and 96 c.c. of salt solution will make the right proportion for the tubes. This will prevent the clotting of the 0.06 c.c. of blood used, and does not in any way interfere with the reaction. If salt solution alone be used, the slight clot which forms detracts from the accuracy of the test.

0.06 c.c. of blood is put in the front tube and immediately shaken. The blood is blown out of the pipette using the mouth piece of a blood counting pipette. 1 c.c. is taken from the front tube for the control tube. The pipette is then washed with salt solution, water, alcohol, ether, and dried in the flame of a Bunsen burner. It is now ready for the next patient. Blood is taken from known positive and negative cases for control.

Add 0.1 c.c. of properly diluted Noguchi antigen to the front row of tubes and 0.1 c.c. of salt solution to the back row.

Add 2 units, usually 0.4 c.c. of 10 per cent complement to all tubes. Shake. Place in water bath at 37° C. for ½ hour.

\*From the State Hospital, Stockton, Calif.

Remove and add 3 units of antihuman amboceptor in 1 c.c. of salt solution. (A unit of amboceptor has the same meaning as usual; i. e., the quantity which will hemolyze 1 c.c. of 1 per cent suspension of washed human red cells in 1 hour with 1 unit of complement.)

Place in water bath at 37° C. for 1 hour.

Compare each front tube with its corresponding back tube. Complete inhibition of hemolysis in the front tube with an average amount in the back tube would be + +. Less than this but more than 50 per cent inhibition would be +. More accurate readings can be made with a colorimeter, using a standard in the wedge and comparing each tube with the standard. Tubes must either be allowed to settle after incubation or they can be at once centrifugated and read.

The amount of red cells used in this test is about equal to 1 c.c. of 3 per cent suspension or 3 times that used in the Noguchi test. This is not a disadvantage, as no more will be hemolyzed than the unfixed complement can manage. The hemolysin is always the same, as none is added with the patient's serum. This might occur if serum and cells came from individuals of different groups, unless the cells belong to group 4 of Moss, as I mentioned in a former contribution.

The advantages of this method are obvious. The blood taking is very simple and relieves the patient of much discomfort. No time is lost in getting clear serum. There is no washing of blood except for standardization of reagents. The test can be completed in 2 hours from the time the blood is taken.

I have found the test to be accurate. I have checked it by both Wassermann and Noguchi results.

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## A MODIFICATION OF GERHARDT'S TEST FOR DIACETIC ACID IN THE URINE\*

BY HARVEY P. BARRET, M.D., CHARLOTTE, N. C.

IN spite of its fallacies, Gerhardt's test for diacetic acid in the urine remains the most reliable and probably the most widely used test for the detection of this substance. The method as described by Webster, is as follows: "To 10 c.c. of urine are added a few drops of ten per cent ferric chloride solution. This is best added as long as a precipitate of phosphates occurs, these latter bodies being then filtered off. To the filtrate are added a few drops more of ferric chloride solution when the urine shows a Bordeaux-red color in the presence of diacetic acid." The disadvantages in the actual performance of this test are: the time required to filter off the heavy phosphate precipitate; the uncertainty as to the amount of ferric chloride solution to be added, sometimes requiring a second filtration to remove all the phosphate precipitate; the difficulty in reading, promptly, a negative reaction; and the inability to detect, with certainty, small quantities of diacetic acid.

The following simple modification of Gerhardt's test is suggested: About

\*From the Laboratory of the Charlotte Sanatorium, Charlotte, N. C.

2 c.c. of the urine to be tested are placed in a test tube and an equal quantity of ten per cent ferric chloride solution is allowed to run slowly down the side of the tube. There is thus formed a distinct layer of urine above the reagent. The tube is held at an angle of forty-five degrees. At the point of contact there is a ring of phosphate precipitate, while just below this a Bordeaux-red color is formed if diacetic acid is present. The color appears immediately and tends to diffuse downward into the ferric chloride solution on standing. It may be allowed to stand twenty-four hours, or the tube may be heated as in the original method for differentiating other substances, which give a reaction similar to diacetic acid. The whitish appearance of the phosphate ring serves as a contrast to the red color and makes the reading of a weak reaction much easier. The ferric chloride solution may be first placed in the tube and the urine superimposed or the ferric chloride solution may be run under the urine from a pipette introduced to the bottom of the tube. The best results, however, are obtained by the method as first described.

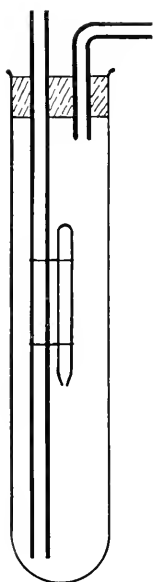
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### PREVENTION OF FROTHING IN AERATION METHODS.\*

BY A. B. DENISON, M.D., CLEVELAND, OHIO.

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WHILE working with the urease aeration method for the quantitative determination of urea, we experienced the common difficulty of foaming in the aeration tubes. We finally hit upon the following device, which absolutely controls the foaming and is very economical in the use of antifoam mixture. It also has an added advantage in that the apparatus does not need to be watched at all during a determination:



\*From the Medical Research Laboratory, Lakeside Hospital, Cleveland.



Glass tubing of 4 mm. inside diameter is cut into sections about 8 cm. long. One end of each section is sealed off in the flame, and the other is drawn to a point with an opening about 2 mm. in diameter. Some means is then provided of attaching this to the long glass tubing in the aeration tube. Wire clips are easily made that serve the purpose admirably. This short tube is then completely filled with the reagent used to prevent foaming, e. g., amyl alcohol, and it is attached as shown in the figure. The foam rises in the tube until it reaches the capillary tip, when enough alcohol leaves the tube to lower the surface tension sufficiently so as to prevent foam rising higher. Very little alcohol is required. It may be necessary to vary the size of the capillary tip with various chemicals used to prevent foaming.

Experiment shows the amount of ammonia caught in the sealed tube as the alcohol is drawn out is negligible, and does not affect the determination.

This device is applicable to all aeration methods in which foaming occurs.

A small wash bottle (filled with alcohol), having a very fine capillary tip, makes the filling of the sealed tube very simple.

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## EDITORIALS

### *The First Year of the Michigan Tuberculosis Survey*

IN a former article in this journal (vol. i, p. 134) I told of the plan of the Michigan State Board of Health for carrying out the tuberculosis survey of the state, a work rendered possible by the appropriation of the sum of \$100,000 by the legislature of 1915. At the time of my former writing the survey was in its beginning. Now, one year has been given to it, and it may be of interest to give in some detail the plan pursued and the results so far obtained. The purposes had in view were: (1) The finding of, by physical examination, so far as possible, all cases in the state. (2) The instruction of each tubercular individual, so far as found, and of his family in the methods of living best suited to prevent the spread of the disease to others and to arrest the progress of the disease in the individual. (3) The placing of each infected individual under the care of a local physician. (4) The awakening of each community to the desirability of concerted action in the endeavor to restrict the disease.

The area unit of the work has usually been the county, and the time devoted to each county has been three weeks. In some of the more sparsely inhabited sections two counties have been taken as the unit. During the first week two visiting nurses go into the county and, first of all, they visit the local physicians

and secure their cooperation. This has been most successful and no physicians have failed in giving the work most hearty support and personal aid. The nurses then go to the teachers and ask them to advise all scholars who seem under par in health to come to the clinics. Along with the nurses, the publicity agent goes. He secures the cooperation of the local newspapers which print articles furnished by the agent, and advertise the free clinic and advise all to go to the clinic. Thousands of columns of information concerning tuberculosis have been published by the newspapers of the state without cost. Ministers are asked to preach about tuberculosis on the Sunday at the close of the first week. Manufacturers are asked to advise all men working under them, and who are in any way out of health, to go to the clinic.

The second week is the clinic week. The diagnosticians are selected for their special skill in the detection of the disease in its early stages. They are men who have the respect and confidence of the profession. Clinics are held simultaneously in each important city or village in the county. In each place three rooms are used, a waiting room, a dressing room, and a clinic room. In the first are trained nurses taking the histories and the temperatures, and making a triplicate record. These records accompany the patient into the clinic room where the expert makes his examinations in the presence of the local physicians. Indeed, he gives a clinic on the diagnosis of tuberculosis. It is estimated that more than ninety-five per cent of the local physicians attend these clinics and some have become so deeply interested in the work that they have followed the expert from county to county, and have become so skilled themselves that they have been added to the staff. The clinician continues the records begun by the nurses, and turns over one copy to the family physician of the individual, who is expected to report the further history of the case.

The third week is given to educational work and this is devoted to both the community and to the individual. Every positive case found by the expert is visited in his home by a trained nurse who gives practical lessons in house sanitation, food, sleeping quarters, etc. Talks are given in the schools, factories, churches, clubs, etc. The county legislators, or supervisors, as they are called in this state, are seen and the county's needs and duties are pointed out. The local physicians are urged to provide weekly free clinics for the detection of tuberculosis. The need of a county sanatorium for the cure and education of the positive cases is made evident. In all these directions, the work is bearing excellent fruit. As a result of this work, seven full-time health officers have already been provided in small cities and several counties have made appropriations for sanatoria.

I am quite convinced that the training of the physicians of the state in the early diagnosis of tuberculosis will ultimately prove of the greatest value. The people in general will soon forget about the campaign against tuberculosis, but the local physicians will not forget and with their weekly free clinics they will keep the work going, will prove its value to the community, and will secure for themselves a higher appreciation of the nature and value of their services.

As concrete examples of what is being accomplished, I may mention St. Clair and Ingham Counties, of which Port Huron and Lansing are the county seats. In the former county 464 persons were examined and 86 found to be

positive and 82 suspicious. These findings have led already to provision for an open air school for the tuberculous children, a full-time and permanent visiting nurse, and a vote to appropriate \$15,000 for a county sanatorium. In Ingham County 524 persons were examined with 116 positive, and 97 suspicious. As a result, the county has a sanatorium, and the city, a full-time health officer.

Among the school children examined, 1114 have been found to have the disease. However, a larger number has been found in the productive age—from twenty to fifty years.

The total population of the area surveyed in the first year is 1,319,283. The number examined is 11,528; positive, 2,914; suspicious, 2,231; arrested, 375. There is no claim that all cases of tuberculosis in the area covered were found. The physicians in their weekly clinics are constantly adding to the number. It would not be fair to suppose that if all the inhabitants of the area were examined the percentage of positive cases would be nearly so high as that found among those actually examined, because these were selected for examination either because they were known to have been unusually exposed to this infection or because of the evident fact that they were not in robust health. Of the positive cases found only two per cent had already been reported. In other words, ninety-eight per cent were cases which had not been recognized until this examination. The positive cases are distributed by stages of the disease as follows:

Advanced .....	6.8 per cent
Moderately advanced .....	27.2 per cent
Incipient .....	52.6 per cent
Arrested .....	12.9 per cent
Not determined .....	0.5 per cent

The medical history of this year's work is now being prepared and will undoubtedly make a valuable contribution to the literature of tuberculosis. It is the hope of the State Board of Health that it be permitted to go over the state again in a similar way and finally so organize each county that it may efficiently continue the work until the disease is wholly eradicated.

—V. C. V.

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### *The Choice of Ruling in a Blood Counting Chamber*

THE introduction of the blood counting apparatus with the well-known ruling of Thoma has been of inestimable value to clinical medicine. So indispensable is it that the physician of the present day cannot practice modern medicine without a satisfactory hemocytometer as part of his equipment.

The original Thoma ruling of the counting chamber is designed for enumerating the erythrocytes alone. It is not, however, a satisfactory ruling for counting the leucocytes. To secure a ruled surface in a counting chamber adapted to enumeration of *both* red and white cells, the original Thoma ruling has been modified; among the best known modifications are the Neubauer and Türk rulings. Of the various rulings described, that of Neubauer is superior to all others, in

the opinion of the writer. The ruling for counting the leucocytes is simple and employs the fewest possible lines for an accurate count.

So obvious is the advantage of the Neubauer or Türk ruling over the original ruling of Thoma, that it is unfortunate that the latter is still upon the market. In protesting to one of the well known dealers in this country against the sale of counting chambers with the Thoma ruling, the writer was surprised to learn that *more than one-half of all orders received, specified the Thoma ruling*. This is doubtless due to ignorance on the part of the purchaser, for the cost of a counting chamber with one of the newer rulings is very little more.

To do away with the sale of counting chambers with the original Thoma ruling, it is to be hoped that authors of works on clinical pathology will eliminate from future editions illustrations of the Thoma ruling, showing only those of Neubauer, Türk, Zappert-Ewing, etc. It would also be a great gain, if the counting chamber manufacturers and dealers would agree to discontinue cuts and descriptions of the original Thoma ruling in their circulars.

The counting chamber designed by Bürker in 1907 is rapidly growing in popularity. It provides two ruled areas, and preparations for counting both red and white cells can be made at the same time, thus shortening the operation considerably. Filling the chamber is also much simpler in the Bürker hemocytometer. A disadvantage of Bürker's chamber, the writer believes, is the ruling. Several years ago, at the request of the writer, Zeiss made the Bürker chamber with the Neubauer ruling; and very recently an American firm, Max Levy, has also placed the Bürker chamber with Neubauer ruling on the market, as well as a modified chamber with various rulings.

Those purchasing counting chambers are urged to investigate the advantages of the different rulings before ordering apparatus. Authors and dealers might render a service by emphasizing the fact that the *Thoma ruling is suitable for counting red blood cells only*.

—R. S. M.

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### *Vitalism and the New Physiology*

IN the ever expanding volume of biological literature there occasionally appear articles in which an account is given of the present-day attitude of different schools of thought regarding the time-honored question as to the essential nature of the life processes. The well-known address of Sir Albert Schäfer before the British Association in 1912, that of Sir Oliver Lodge two years later before the same society, and still later, in 1916, that of Professor A. B. Macallum<sup>1</sup> before the Society of Biological Chemistry of this country, have served during recent years as bulletins of the progress that is being made. If we were to take these three papers as the sole evidence upon which to form an opinion, we should be compelled to conclude that a mechanistic conception of the life processes is more tenable than one which holds that some specific

<sup>1</sup>Presidential address, Jour. Biol. Chem., 1914, xvii.

influence, called for want of a better term "vital force," is the important controlling factor. But again we must reopen the controversy, for in his recent Harvey lecture entitled "The New Physiology," Dr. J. S. Haldane of Oxford,<sup>2</sup> England, has presented a mass of evidence which is so strong in favor of a view which, although not vitalistic in the older, narrower sense, is so definitely opposed to that of pure mechanistic physiology that it cannot be disregarded.

It is pointed out at the start of this lecture that it was about the middle of last century that a group of physiologists, impressed with the wonderful advances then being made in physics and chemistry, broke away from the "vitalism" of their fathers and essayed to demonstrate, by their experiments, that all animal functions are explicable on a purely physico-chemical basis. Although it is undoubtedly the case that great success often attended the researches undertaken from this point of view—it being often possible to find some satisfactory physico-chemical explanation for an isolated physiological process—yet as time has passed, fewer and fewer have been the results of such a nature, until at last we have come to recognize that to obtain them, innumerable conditions in the environment of the responding tissue must be maintained in a so-called "normal state." Now, as Haldane insists, the necessity for the existence of this normal state, in order that a given stimulus may always have the same effect, indicates that there is something involved in the life processes that is not present in the physical world. "The physiologists who led the revolt of last century against vitalism" did not see the network of conditions which come into play in influencing the effect which a given stimulus will have, and moreover these older workers started out from a false physico-chemical viewpoint in that they imagined the then recently discovered fundamental laws of chemistry (the atomic theory, etc.) to represent the bed-rock of physico-chemical knowledge.

The maintenance of this normal state is the one characteristic of living things, and the aim of the new physiology should be "not to ascertain the causes of vital activity but to trace out its normal details." To make this clear, various examples are given of recent researches, particularly of those in which the author has himself taken so prominent a part. Thus, in the response of the respiratory center to the slightest changes in the  $\text{CO}_2$ -tension of the blood is seen a mechanism of the finest order of delicacy for maintaining a normal reaction, that is, a normal H-ion concentration, in the blood. Whenever anything occurs which tends to upset this normal condition, causes, such as stimulation of the vagi, come to have effects which are entirely different from those ordinarily observed: e.g., apnea cannot be produced by ordinary inflation or deflation of the lung when there is a high tension of  $\text{CO}_2$ , but it is readily produced when the tension is low.

As another illustration, take the recent observations by Haldane and Priestley on the excretion of water by the kidneys. After drinking very large quantities of water the urine becomes extremely copious and very dilute, thus indicating that the secreting cells of the kidney are stimulated to activity by some change in the concentration of the blood; they form one of the physiological mechanisms involved in maintaining "the normal." The question is, must a

<sup>2</sup>Reprinted in Science, Nov. 3, 1916.

physico-chemically measurable change in blood-concentration occur to bring about this diuresis, or are the cells so reactive to deviations from the normal that they respond to changes in concentration which we cannot detect? To answer these questions, the authors examined the hemoglobin content and the electrical conductivity of the blood, before and during the potations, with the result that no change could be detected in hemoglobin content, and one that was just perceptible in electrical conductivity. Evidently then the renal cells, like those of the respiratory center, are attuned to react with marvelous sensitivity to changes from "the normal" in the blood which are no more than just perceptible by the most delicate and refined of physico-chemical methods of measurement.

These observations along with many others, such as the constancy in the concentration of sugar and sodium chloride, indicate that a very fine physiological regulation of the composition of the blood is provided, which is also the case for the regulation of its total volume and temperature. These are the "normals" and the function of the physiologist should be not to seek for some causal explanation for each separate normal which he may succeed in isolating and studying apart from other normals, but to seek for interconnected normals and "their organization with reference to one another and to other organic normals." This is not vitalism but simply biology, being the same method of study as that followed by the anatomist "who seeks for the normal—the type—which runs through and dominates the variety of detail which he meets with, and who reaches more and more fundamental types." Such an idea of an organized normal, as the determining factor for physiological reaction, brings "unity and light into every corner of physiology, for it helps us to predict just as the ideas of unalterable mass and energy help us to predict." Life then is something apart, it is an entity, and it cannot be defined in terms of anything simpler, "just as we cannot define mass or energy in terms of anything simpler."

The new physiology, therefore, concerns itself with the study of the normals, how they are related to one another, how they react on or are affected by other normals, their relationship to environmental conditions, and so forth. It is not biophysics or biochemistry, but is biological physiology. The attempt to interpret living organisms in terms of physico-chemical laws is judged as having been "the most colossal failure in the whole history of modern science."

The paper concludes with a comment on the relationship between physiology and medicine, which in essence is to the effect that "the mechanistic physiology of the nineteenth century has failed to take the rightful position of physiology in relation to medicine." The reason given for this false attitude is the failure of the mechanist to recognize that in life we have to deal not with simple matters of cause and effect, but with interconnected normals. Disease is a perversion of the normal, so that the physician must know the normal in its elastic and active organization; he must know how health is maintained under the constantly changing conditions of environment. His pathology should concern itself largely with the study of how health tends to reassert itself under abnormal conditions, and his pharmacology should be not so much an investigation of the action of drugs, but how these may be used to aid the body in the maintenance or reestablishment of health. The author claims to be able to see this new physiology as applied to medicine growing up more quickly in this

country than in the old, because we have the advantage of having less of old intellectual machinery to discard.

In advocating that the physiologist in his investigations should proceed, without any bias in favor of a physico-chemical hypothesis, to collect accurate information regarding the working and interdependence of the various functions which go to make up the normal, there is no clear argument that a mechanistic view of life is untenable. That the normal condition of the blood should require for its maintenance a degree of sensitivity on the part of the respiratory center (toward H-ion concentration) or of the kidneys (towards the water content of the blood) that is reactive to changes which we can scarcely recognize by physico-chemical means surely does not warrant us in denying that a mechanistic interpretation for the reaction may exist. So far as any conclusion is justifiable, it would appear to be merely that the reactive tissues are of a delicacy that is greater than the physicist or chemist is at present familiar with. Nevertheless the lecture is of immense value in warning us that we should adopt, for the present at least, a different attitude towards many biophysical and biochemical problems, and, instead of going out of our way to explain them in terms of some mechanistic hypothesis, devote our attention to accurate and thoroughly controlled observation of the facts.

—J. J. R. M.

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### *Epilepsy*

EPILEPSY has for many years been a puzzle. Doubtless it will remain a puzzle judging from the remark of Foster Kennedy<sup>1</sup> that "from the West we hear of the epilepticoccus and the removal of the intestine, and from the East we are told that epilepsy represents an effort of the individual to resume his fetal position in the mother." If we come no closer to it than the limits set in that statement, no wonder we worry.

Also in view of the appearances of various dogmatic statements concerning the causes of epilepsy in each of which there is a grain of truth—and sometimes two grains—we should be grateful for a discussion, which, like Dercum's,<sup>2</sup> summarizes fairly the situation. Dercum calls attention to the fact that, as has long been known, heredity plays a part in the appearance of epilepsy, and, as he says, if one considers all forms of nervous and mental disease in the ancestry, then the heredity percentages are high. If the inquiry is restricted to the transmission of epilepsy alone, the percentage falls. But even so a family history of nervous and mental diseases is of great significance. Other factors than heredity are alcoholism, syphilis, and other toxic factors among which is lead poisoning, each of which, when it is an ancestral factor, acts through the germ plasm in establishing an unstable individual, and so, Dercum says, "approaching the subject from whatever point of view we choose, the inference is unavoidable that in a large number of epileptics there has been a primary, a basic impairment of the germ plasm. Such an impairment may show itself in various nervous or mental conditions, among which is epilepsy."

<sup>1</sup>Kennedy: Jour. Am. Med. Assn., 1916, lxvii, 252.

<sup>2</sup>Dercum: Ibid., 247.



Aside from these cases there are others in which there can be no reasonable doubt that the disease is directly produced by intoxications and infections. As intoxicants, alcohol and lead are known. As infectious agents many of the bacteria are known to be active. The convulsions which accompany the infectious diseases of childhood are to be regarded merely as epiphenomena of the infectious processes, and are to be explained by a direct toxic action on the cortex. Quite commonly they disappear with the infection. Unfortunately, they occasionally persist as established epilepsies. Epilepsy every now and then follows an attack of typhoid or scarlet fever, less frequently measles, whooping cough or influenza. Not uncommonly there is a history of convulsions accompanying the infection, and then, after an interval, possibly of months or of years, the establishment of epileptic attacks follows. In such cases the cause may be in mild encephalitides or even in scarring and contraction of the meninges caused by the original infection.

Another factor is trauma, which produces mild lesions at the time of injury, which in healing lead to irritation of the cortex.

When, Dercum says, we review the facts, one fact stands forth with striking prominence, namely, that epilepsy is not a specific clinical entity, but is a symptom complex which may be brought about by one of several chance factors. Whether the cause in a given case is internal secretional defects, infection or trauma, one may only discover by close analysis of the case. Treatment will be governed by these discoveries.

—P. G. W.

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### *Anatomy from the Clinical Standpoint.*

THERE is probably no part of the description of the body so badly in need of revision in our textbooks as that of the pelvis, and this notwithstanding the fact that many attempts have been made during recent years to elucidate further the problems connected with this region. The old descriptive anatomy is rapidly becoming superceded and the body is being viewed as indeed it must be if the physical features in their details are to have any real practical and living value, from the standpoint of function. This by no means belittles the significance of pure morphology. It simply brings anatomy into line with the practical study of medicine from which during the last two decades it has tended somewhat to become divorced.

Dissection, while it reveals the details of structure so necessary for adequate knowledge of the body, is apt by the very technic employed to obscure our view of the body as a functioning structure. We fail to get the correct perspective; to estimate properly the proportionate importance in function of the different elements. As an example we may quote the extraordinary ingenuity involved in framing the description of the pelvic fascia as it is given in all our present day textbooks. So involved is this description and so impossible to comprehend that on the face of it we are inclined to doubt if the description can accurately represent the actual conditions. So misleading has the time honored statement of the pelvic fascia become even to anatomists of standing that we find in the present

edition of Cunningham's "Practical Manual" the curious statement that the bladder prostate, lower part of rectum and ureters are not in the pelvis at all, but in the pelvic wall since they are imbedded in pelvic fascia. Surely when descriptive anatomy has attained such ludicrous extremes it is time to call a halt.

Many writers working on the clinical aspect of anatomy have added greatly to our knowledge of the functional significance of the structures in the pelvis, more especially the importance from a practical standpoint of the connective tissue which is massed together under the general term *pelvic fascia*. So far they have been unable to influence the textbooks, largely because they have confined their remarks to isolated portions of the pelvis and have not dared boldly to overthrow the time honored descriptions.

If we are thoroughly to understand the significance of the pelvic connective tissue, it is not sufficient simply to "depolarize" the terms employed; these have been used for so many generations that it is not possible to eradicate from the minds of readers their ancient significance. It is necessary to describe the pelvis entirely anew, using terms which shall have a fresh significance and which cannot by any possibility be misunderstood. Until this is done ruthlessly and efficiently, anatomists must expect that their clinical colleagues will continue to deplore the unpracticality of the study of conventional anatomy.

Let us consider for a moment the connective tissue surrounding the vessels and nerves which run to the rectum entering the viscus below the great valve of Houston, in other words, through the perineal chamber. Quenu and Hartmann in their description of resection of the rectum stated that after cutting through the levatores ani the surgeon will find the sacro rectal fibrous tracts and certain insignificant nervous and vascular branches which he will tear out or cut through to free the rectum. Naturally it is not here the purpose to discuss the precise method of excision of the organ. We wish merely to emphasize the fact that our brilliant French contemporary was misled by conventional anatomical description. It is true that the connective tissue when dissected is not powerful but is it right to consider this tissue, as many anatomical writers do, in the wholly artificial state of being separated into sheets or strands postmortem? Every surgeon realizes that when he has cut the levatores ani only he can pull down the rectum a matter of two inches at the most and that if he stitches the bowel into the anal skin there will be considerable tension on the sutures. There must be something holding up the rectum and this something is really the "insignificant" strands of connective tissue surrounding the vessels and nerves of supply.

The term rectal stalk has been suggested by Professor Elliot Smith for the perineural and perivascular connective tissue supporting the perineal chamber of the rectum, in the conventional description the lateral part of the lower or rectal layer of the endopelvic or visceral pelvic fascia. The conventional term, however, means nothing and should be discarded; it should be replaced by Waldeyer's term, fascia propria of the rectum, since it is desired to convey the significance that this fascia clinically makes the rectum a "self-contained" organ. Laterally the fascia propria is more extensive, constituting the rectal stalks.

That these rectal stalks are actually of importance in supporting the rectum is obvious from our surgical experience already mentioned in the old operation of perineal resection and if this operation is now largely discredited it has at least taught us the necessity for revising our description of pelvic anatomy. In a series of dissections made upon new born infants and upon adults I found that the isolated but undissected rectal stalk measures only about half the length of the contained nerves and blood vessels.

The nerves and blood vessels therefore are not made use of as supporting bands; one would not expect it; the surrounding connective tissue in bulk mingled with the accompanying muscular strands acts as the real but quite elastic support of the viscus. The comparative length of the nerves and blood vessels allows some possibility of movement of the rectum as a whole, permits prolapse to a limited extent, as seen in infants, but so long as the nerves and blood vessels remain uninjured the connective tissue surrounding them allows only a moderate displacement.

Relatively speaking the rectal stalks in the infant are as long as those in the adult and it is not correct to state that prolapse in the young child is due to laxity of the attachments of the organ. Prolapse is permitted in the infant, as it would be in the adult, but for the fact that in the latter the sacral promontory overhangs and protects the rectum from direct pressure. Moreover in the adult the genital and vesical organs are not, as they are in the infant, in a position to exert pressure on the rectum.

We are not therefore to imagine that the supports of the rectum are in any way rigid or inelastic. But we must also not imagine on this account that the supports are indefinite or inadequate. We must in other words look upon the anatomical relations and facts from a new standpoint if we are to interpret their significance with any degree of assurance.

I have merely used the rectal stalks as illustration of the newer conceptions which are presenting themselves to anatomists and to clinicians alike in the study of the human body and I venture to predict that with the spread of these conceptions anatomy will become the study of the living body, it being recognized that the cadaver presents only a gross caricature of the functional conditions represented during life.

—*T. Wingate Todd* (per *J. J. R. M.*).

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### *Spirochetes in Healed Syphilis*

“**B**ETWEEN the pathologist and the clinician there is always a certain antagonism of attitude with reference to the curability of any given disease. The clinician is, and naturally must be, more or less optimistic as to the results of therapy. . . . The pathologist . . . becomes pessimistic as to ultimate cure because of his constantly recurring experience of finding evidences of active disease in cases clinically cured.”

With this statement Warthin<sup>1</sup> introduces an account of his study of a series of clinically cured cases of syphilis in which he sought the *Treponema pallidum*. He used the original Levaditi method, applying it in 41 autopsy cases which were examined on the pathologic service of the University of Michigan. These cases were divided into three groups: (1) cases in which there was a known history of syphilis, with treatment regarded as adequate and resulting in a cure; (2) cases in which the syphilitic infection was recognized as still active, treatment being continued; (3) cases in which a syphilitic history could not be obtained or was specifically denied by the patient, the clinical diagnosis not including syphilis, and no antisymphilitic treatment given. In group 1, there were 11 cases; in group 2, 5 cases; and in group 3, 25 cases. In all of these, lesions of active syphilis were found and in them the spirochetes were demonstrated. Active luetic lesions were found in the heart in 36 cases, in the aorta in 32, in the testes in 31, in the liver in 4, in the adrenals in 6, in the spleen in 1, in the pancreas in 6, and in the central nervous system in 5. The order of organic infection according to frequency is aorta, heart, tests, adrenal, pancreas, nervous system, liver and spleen. It happens logically then, that the triad of interstitial myocarditis, aortitis, and orchitis fibrosa may be taken as a pathologic complex indicating the occurrence of a syphilitic infection in the male.

Warthin's material comes almost without exception from the University Hospital, an institution in which the patients represent the average middle class population of the State of Michigan, and the forty-one cases he has studied represent about one-third of the adult cases which were subject to postmortem examination in 1912-1914.

In group 1, there was one positive Wassermann, 5 negative, and in 5 cases a Wassermann was not done. In group 2, there were 4 positive Wassermanns, and in 1 no Wassermann was done. In group 3, a Wassermann was not made in 11 cases, was negative in 10, and positive in 4.

It is true, as Warthin says, that the sociologic importance of this is very great. It places latent syphilis upon a plane of importance nearly, if not equally, that of tuberculosis, as a factor opposed to the health and progress of the race. Latent syphilis, Warthin believes, will be found to be the chief factor in the production of myocardial insufficiency and the cardiovascular renal complex, apparently so rapidly increasing—so rapidly, one may add, that an Association has recently been formed to study it and prevent the increase.

From the therapeutic side the lesson is evident, and from the standpoint of serologic reactions, it may be suspected that a negative reaction means nothing.

—P. G. W.

<sup>1</sup>Warthin: Amer. Jour. Med. Sc., 1916 (1153) 508.

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## ORIGINAL ARTICLES

### THE APPLICATION OF THE TYPHOIDIN TEST IN A GROUP OF NURSES AND PHYSICIANS\*

BY FREDERICK P. GAY, M.D. (UNIVERSITY OF CALIFORNIA) AND  
ALBERT R. LAMB, M.D.

THERE seems little reason further to doubt that the skin sensitiveness to preparations of the typhoid bacillus (typhoidin) described by Gay and Force<sup>1</sup> in typhoid vaccinated individuals and in typhoid recoveries is to a large extent specific. Their findings have been essentially confirmed by Pulay,<sup>2</sup> Mehler,<sup>3</sup> Nichols,<sup>4</sup> Kilgore,<sup>5</sup> and Kolmer and Berge.<sup>6</sup> Technical error accounts for the failure of Austrian and Bloomfield<sup>7</sup> to confirm their results.

Question, however, may still exist as to the technic of the test itself and as to the interpretation of the results obtained, and this communication would deal with a separate group of individuals studied from these standpoints. A fuller discussion of all the questions that have arisen in connection with this reaction will appear in a forthcoming article by Force and Stevens<sup>8</sup> with whose work we have been in touch and to whom we are indebted for the improved method applied in this study to a group of one hundred and fifty-four nurses, physicians, and attendants in the Presbyterian Hospital of New York City.

The technic of the typhoidin test as originally described by Gay and Force consisted in the application of a killed, concentrated, old glycerine broth culture of a single strain of *B. typhosus* to the slightly abraded surface of the arm. A positive reaction differed from a negative result in a quantitative difference in millimeters in the diameter of the areola which appeared around the abraded surface, as read after twenty-four hours. Kilgore<sup>5</sup> has attempted with some success to standardize this technic and eliminate the subjective cause of error by establishing a typhoidin quotient. Another improvement consisted in preventing a recognized deterioration in the fluid typhoidin by precipitating and

\*From the Medical Clinic of Columbia University, Presbyterian Hospital, New York.

drying the active constituents by means of absolute alcohol and ether (Gay and Claypole<sup>9</sup>). Of greater significance are the additional modifications introduced by Force and Stevens<sup>8</sup> which are briefly as follows: first, the use of a polyvalent preparation from several chosen strains of typhoid bacilli to allow for the probable varieties and corresponding antigenic differences in typhoid bacilli (Hooker<sup>10</sup>); second, the employment of a carefully determined minimal effective dose of the dried polyvalent typhoidin (0.00002 gm. in 0.05 c.c. of 0.5% carbolated saline); third, the injection of this dose *intradermally*; and, finally, the reading of the reaction in forty-eight instead of twenty-four hours. This later period tends to eliminate the nonspecific irritative reaction which may occur in normal individuals from the proteins of the culture media or the phenol employed as a diluent.

In the adjoining table are summarized the results obtained in vaccinated individuals, typhoid recoveries, and normals by the technic just described. The criterion of a positive reaction consists in the presence after forty-eight hours of a definite indurated papule plus a reddish areola of at least five millimeters. In no instances was a definite papule present without an areola, although the reverse relation was in a few instances the case. No suggestive or doubtful reactions are included, but in any case where doubt existed the test was repeated and any result which did not meet the specified criterion was classed as negative. Repeated tests agree in almost every instance.

SUMMARY OF TYPHOIDIN REACTIONS IN NURSES AND PHYSICIANS  
AT THE PRESBYTERIAN HOSPITAL.

	No. Examined	Positive	Negative	Per Cent Positive
I. Cases giving a definite history of antecedent typhoid fever (2 to 22 yrs. previously)	12	9	3	75%
II. Vaccinated cases.				
Within six months (New York Board of Health Vaccine)	29	19	10	65.5%
Six months to 1 year                      “	50	32	18	64%
One year to 18 months. P. H. vaccine	24	18	6	75%
Two years to 30 months.               “       “	13	7	6	53.8%
Three to five years. Various vaccines.	5	2	3	40%
III. Normals giving no history of typhoid or typhoid vaccination.	21	3	18	14.28%

Great pains were taken to obtain a careful history in those cases listed as typhoid recoveries, and they may be accepted as fully as accurate as most histories of this sort, although in the majority of cases no certain history of blood cultures and Widal's was obtained. On the other hand, in the normals (Group III) care was taken to exclude those with history of any continued fever. There were five individual cases in which the history of previous typhoid was suggestive with two negative and three positive typhoidin tests which are not included in the table.

It will be noted that the majority of the vaccinated cases had been immunized with vaccines supplied by the New York Board of Health, or else prepared in the Bacteriological Laboratory of the Presbyterian Hospital. The Board of Health vaccine, was, we understand, prepared from the well-known

army or Rawling's strain.<sup>11</sup> The Presbyterian Hospital vaccine was prepared from a strain of *B. typhosus* grown there on artificial media for some twenty years, and originally isolated from the spleen at autopsy. In all these instances the dosage and intervals were the same, namely, three doses of five hundred thousand, one million, and one million bacteria, at weekly intervals.

A survey of the percentage of positive results shows that seventy-five per cent of those individuals that had recovered from typhoid gave a positive reaction. The negative reactions bear no relation to the date of the antecedent disease since two cases twenty-two years previously were both positive. From seventy-five to forty per cent of those that had been vaccinated against typhoid also reacted positively and in general in decreasing correspondence with the lapse of time since immunization. The higher percentage with the Presbyterian Hospital vaccine in the group between one year and eighteen months previously may be accidental or may point to a fresher or more antigenic vaccine.

The results obtained in this group of cases correspond very closely to those recently obtained by Force and Stevens.<sup>8</sup> The percentage of typhoid recoveries giving a positive reaction is not, however, so high as in the original communication of Gay and Force,<sup>1</sup> which may depend on a more rigorous criterion of determination, or possibly may be a more correct indication of the degree of protection which is actually afforded by recovery from typhoid fever. It has generally been assumed that recovery from this disease protects in the great majority of cases, and in statistics over long periods of time it has been found by most observers (Curschman,<sup>12</sup> McCrae<sup>13</sup>) that only two or three per cent of cases of typhoid fever give a previous history of the disease. Such figures would seem an overestimate rather than an underestimate, since the period at which these figures were obtained antedated the differentiation between typhoid and paratyphoid, which must have been confused. On the other hand, more recent investigations by Sawyer<sup>14</sup> and Kelly<sup>15</sup> show that under conditions of massive infection from eight to fifteen per cent of the cases of typhoid may give histories of previous typhoid fever. Protection against typhoid, either by recovery or by vaccination must in all cases be regarded as relative, and it is probable that any recovered case of typhoid fever could be infected with the disease if a sufficient dose of living typhoid bacilli were ingested.

The percentage of presumably normal individuals (14.28%) that give a positive typhoidin reaction corresponds closely to the nine per cent originally described by Gay and Force,<sup>1</sup> and the eleven per cent of Gay and Clappole.<sup>9</sup> Such cases are extremely interesting, and the question at once arises as to whether they are individuals with some peculiar nonspecific susceptibility of the skin, or as to whether they may at some time have undergone an unsuspected infection with *B. typhosus*. An apparently well-controlled instance of abortive attacks of typhoid fever in a group which also showed unmistakable, well-defined instances of the disease, all of which gave positive typhoidin tests, has already been referred to by Gay and Clappole.<sup>9</sup> Another possibility is that certain of these normals who react as do typhoid recoveries may be healthy carriers of *B. typhosus*. An attempt to verify this latter hypothesis has recently been made

on two students of the University of California, who gave absolutely no history of typhoid fever and who had not been vaccinated protectively. Both gave a clear-cut, positive reaction to typhoidin. The Widal in both cases was negative in a dilution of 1-10, and an attempt to isolate the typhoid bacillus from the urine and from the stools after elaterin catharsis<sup>16</sup> was negative.

Repetition should be made of the purpose for which this test is employed, as compared with any interpretation of what its true significance may be. Since the reaction occurs in those individuals who are known to be more or less perfectly protected against typhoid fever, the test was originally offered as presumptive evidence of protection in the individual case when positive. We have seen no reason to alter our opinion in this respect, and would point out further indications that this interpretation of the reaction is true, in addition to the simple relationship observed. In the first place, in no individual, in whom a positive reaction has been obtained, has typhoid fever occurred. This might well be due to the relatively small number of such observations that have been made, but it is interesting to find that, on the other hand, in three individuals, who have been vaccinated and in whom a negative reaction was subsequently obtained, typhoid fever shortly thereafter occurred. Two of these individuals were laboratory workers and exposed, and indeed known in one instance to have been subjected to massive infection; the other was a nurse who attended a typhoid patient. In the second place, individuals who react positively, when re-inoculated with typhoid vaccine react much more violently than do individuals who give a negative typhoidin test, in this respect resembling typhoid recoveries, and certainly indicating a reaction and probably protection against *B. typhosus*. And, finally, individuals who have been vaccinated against typhoid and who react negatively become positive in most instances on further immunization. We regard the attempt of Nichols<sup>4</sup> to explain the typhoidin test as an indication of hypersusceptibility rather than of true immunity as supererogatory in view of the fact that both conditions would seem to be coincident.

At all events, whatever may be the eventual understanding of a positive typhoidin test, use of its absence as an indication for re-vaccination certainly errs on the side of safety in detecting those individuals who in spite of typhoid vaccination do not show indications of a reaction to the typhoid bacillus, and who are, therefore, presumptively those particular individuals who under ordinary conditions of infection will be found to be the least protected ones.

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## CARDIAC ANEURISMS\*

BY PAUL G. WOOLLEY, M.D., CINCINNATI, OHIO.

CARDIAC aneurism is a subject I have chosen because the condition is relatively uncommon, and rarity introduces a certain modicum of interest that one may not feel in a discussion of the plain ordinary aneurisms which are more frequently seen. It illustrates the whole subject of aneurism so that in speaking of it one may apply the principles involved in vascular aneurisms, and therefore the interest in the less common condition may be carried to the more usual one.

The heart is after all merely one section of the vascular tree in which arrangements have been made for a certain temporary storage of blood, and in which the mechanism for propulsion of the blood has been developed. Accordingly the possibilities of dilatation have been increased and the contractile tissue of the walls has been augmented to meet the physical demand of forcing a large volume of blood against a constant pressure. Moreover, in order that the blood flow shall prevail in but one direction, the valves, which are endocardial reduplications, and which correspond to the valves of the veins, are highly developed. Structurally, therefore, the heart and the aorta, in which aneurisms are most frequently seen, (not to speak of the other parts of the vascular system) differ from one another merely in the amount of muscle on the one hand, and elastic tissue, on the other. The physiologic activity of the heart, therefore, depends largely upon the quality and quantity of the myocardium, while that of the aorta depends largely upon the quality and quantity of the elastic tissue. The quality of any tissue depends upon the amount of nourishment it receives; which means, as a rule, the amount of normal blood supplying it. The amount of blood arriving at any point of a tissue depends upon the pressure, within or upon, the organ, and upon the condition of the smaller vessels carrying the blood. In ileus, the muscular wall of the gut becomes paralyzed largely because the dilatation (the internal pressure) cuts down the blood supply. In decubitus the skin sloughs

\*From the Pathologic Institute of the Cincinnati General Hospital and the Mary M. Emery Department of Pathology of the University of Cincinnati. Read at a meeting of the Pathologic Section of the Lucas County Academy of Medicine, Dec. 8, 1916.

because external pressure cuts off the blood supply. Degeneration and necrosis take place in acute inflammatory foci because the tissues between the smaller vessels swell and compress the vessels, or, in more chronic cases, because overgrowth of fibrous tissue takes place; and this, in its development and later contraction, compresses the vessels.

In the heart the course of events is roughly as follows: The infecting organisms—in syphilis, the spirochetes—arrive by way of the blood stream and enter the smaller vessels of the myocardium from which they pass into the perivascular spaces. Here they set up an inflammatory change of a chronic order which is characterized by accumulations of small round cells, and a slight edema of the connective tissue cells and of the myocardium. The sequel of this small celled infiltration is perivascular fibrosis which is associated with a minimum of myocardial dégeneration. As time goes on two things happen. First, the perivascular connective tissue becomes well-formed and contracts, limiting the flow of blood through the vessels; and second, it becomes, because of its increased thickness and concentrated colloidal character, less permeable to the dissolved food stuffs of the blood. The result is that the food supply of the myocardium is gradually diminished and atrophy occurs. Then, because of the relative lack of contractile tissue, strain is placed upon the remaining fibrous tissue which is apt to undergo hypertrophy, and so fibrosis appears. Each area of fibrosis represents a point of weakness,—a point at which the wall of the heart is less resistant to continued pressure. In the aorta the same process commences about the vasa vasorum and it is this that accounts for the characteristic pearly, succulent, raised patches, and for the intimal puckerings. Sections through these pearly patches show an intact endothelium, a hyperplastic subintimal connective tissue, (the result of strain, I take it), an atrophied muscularis, and a degenerated disappearing elastica, and about the vasa, which supply the outer two-thirds of the vascular wall, well marked small round cell infiltration. From this time on the changes are degenerative. These terminal degenerative changes are the ones which produce the appearances which are indistinguishable from those associated with the ordinary ones of the so-called senile atherosclerosis. They are developed because of interference with the nutrition of the scar tissue in exactly the same manner as the primary degeneration of the media. The course of events is as follows: The normal aortic wall is nourished in its outer two-thirds by a direct vascular supply. The inner third receives its nourishment by indirect means, from the lumen by absorption and from the outer vascular layers also by absorption. Therefore, when medial and subintimal fibrosis has developed to a certain degree, and especially when the blood supply to the outer two-thirds has been reduced as we have described, a relative lack of “food” is developed and the newly formed tissue tends to die, in evidence of which, what one knows as lipoid and fatty degeneration occurs, and atheromatous abscesses appear, which, as time goes on, either become ulcers, or, becoming calcified, form plaques.

There are two periods during which the vessel wall or the myocardium, as the case may be, is particularly weak; i. e., during the period of more acute inflammatory change, and during the period of degeneration. At either period an aneurism may develop provided the physical conditions (i. e., internal pres-

sure) are propitious. Usually, during the latter stage which appears relatively late, the individual is less active and the internal pressure is relatively low. Aneurisms are, therefore, less frequent in old age. On the other hand, during the early stages, the individual is active, and apt to be subjected to physical strain.

This factor of strain, while it seems to be a secondary factor in the production of aneurisms, also seems to be a necessary one. The primrose path by itself does not conduce, of itself, to aneurism, but as a person follows it, it seems that he must stop occasionally and do some hard work if he is to have an aneurism. This is why it is the young men, men from 30 to 45, who are the sufferers from this condition. In my series of 24 aortic aneurisms, 14 were in patients under 50 years of age; 20, under 60 years. In a series of 12 diffuse aortic dilatations, 10 were under 60 years of age. In a series of eight perforating aneurisms one was 27 years old; one, 35; two, 37; one, 40; one, 42; and two, 43. In the present series of cardiac aneurisms, one was 35 years of age, the other four were in the early fifties. In these series, if one makes allowance for the time during which the lesion has been developing, one sees very clearly that aneurism is a disease of years preceding middle age, the time of greatest physical activity.

These are briefly the facts of arteriosclerosis. They have been applied less generally to the heart. Nevertheless they do apply to the heart fully as well as to the vascular system. Let us summarize them as follows: Just as long as the blood supply remains adequate a tissue will do its work within physiologic limits, even if the demands are very great. A heart will hypertrophy just as long as its blood supply (through the coronaries) is sufficient. It will dilate as soon as the blood supply is not adequate. A hypertrophic heart is one which is a well heart; a dilated heart is a sick one. It frequently happens, of course, that the parenchyma of an organ is not able to make use of an available food supply. When this happens it is because the composition of the blood, aside from the nutritive constituents, is abnormal and this reacts upon the muscle to damage it. The abnormal materials in the blood are sometimes soluble and diffused substances (like toxins, phenol, etc.), and then the effects upon the organ are diffuse ones: they may also be discrete, and insoluble (emboli, bacteria, etc.) and then the effects are focal.

So much by way of an introduction.

\* \* \* \* \*

Cardiac aneurisms bear somewhat the same relation to diffuse cardiac dilatations that vascular aneurisms bear to vascular dilatations. These vascular lesions are most prominent in the aorta, which, if it be affected focally, becomes the seat of local dilatations (aneurisms) and if diffusely damaged, becomes dilated. If a heart is diffusely damaged it dilates as a whole; if focally damaged, it develops a focal dilatation.

Cardiac aneurisms may be acute or chronic. In the acute cases, the dilatation is rapid and definite and depends upon the appearance of areas of softening in the myocardium which are the result of degenerative processes caused by sudden cutting down of the supply of nutriment to the myocardium with or without the presence of bacteria. These sudden vascular effects are the results

of the appearance of thrombi or emboli in the branches of the coronary vessels. Emboli may come from infected valves or from primary seats of disease in other parts of the body. When such sudden degeneration or necrosis (infarction) of the myocardium appears, the cardiac wall is weakened and bulges under the influence of the intracardiac pressure, and, in case the bulging is sufficient, the myocardium ruptures, and hemorrhage into the pericardium occurs. In some cases, because of limitation of the dilatation by pericardial adhesions, rupture does not take place, and if time and infection permit life to persist, the bulging becomes permanent.

On the other hand, well-formed chronic aneurisms result as a rule from focal myocardial fibrosis, caused by chronic coronary circulatory defects, or chronic myocardial infections.

Myocardial fibrosis occurs as a diffuse, or as a focal, process. In the first case it is most frequently, perhaps, a sequel of myocardial overwork, as, for instance, in persons in whom there exists peripheral vascular sclerosis and high blood pressure. It also occurs as a somewhat diffuse process but with a tendency to focal arrangement as a result of certain infections, as, for instance, syphilis, and acute rheumatic fever. It is possible that it is a sequel of other infections which run a subacute course and which are septicemic in character. In the second case it follows the focal myocardial infections, or localized degenerations, such as those which occur as a result of coronary sclerosis.

Chronic cardiac aneurisms result from focal myocardial fibrosis because at the seat of a myocardial scar the heart wall is weakened, not so much because fibrous tissue is weaker than muscle, which it is not, but because it has no "come back"—it is not contractile as muscle is—and so, being stretched by the force of the blood pressure, it tends to remain stretched, and, in the course of time, to be more stretched until it forms a saccular continuation of the cardiac chamber. These aneurisms have a predilection for the anterior wall of the left ventricle, near the apex, a region supplied by the anterior or descending branch of the left coronary, the vessel which supplies the anterior papillary muscle of the left ventricle and the interventricular septum. Since acute complete closure of the artery leads to sudden death of the myocardium, it is apparent that for the production of a fibrosis, the vessel must be gradually changed, as by a developing sclerosis; or the myocardium may be scarred in the course of healing of an acute lesion caused by a small infected embolus which does not block enough of the main vessel to bring about an immediately fatal result.

After the formation of a myocardial aneurism has begun, adhesions between the heart and the pericardium may occur, and the dilatation may be hindered, or, what happens less frequently, the heart may rupture into the pericardium. If there are few or no pericardial adhesions and rupture occurs, death may ensue rapidly; if there are many adhesions, death may be postponed or may occur from some other cause. Also, as in other aneurisms, the sac may become filled with thrombi and prevent rupture, and also the endocardium may become tremendously hypertrophied (fibrosis) and so prevent rupture. "Weeping," or oozing of blood through the wall is not, however, prevented in such cases. Many of the cases of spontaneous rupture of the heart are in reality cases in which the break has occurred in the wall of a cardiac aneurism.

The following cases illustrate the subject of cardiac aneurism:

CASE I.

M. P., No. A-2775, a woman of middle age, was brought to the hospital on May 20, 1916. When she arrived in the receiving ward she was moribund, and died 10 minutes later. No history was obtained.

AUTOPSY PROTOCOL.—The body was that of a short, thick woman, well formed and well nourished. Rigor mortis was not present; postmortem lividity was present. The whole body, above the waist, was covered with scratch marks and in the axilla and the hair of the head there were tremendous numbers of pediculi and ova. Pediculi were crawling on the surface of the body. When



Fig. 1.—Case A-2775. (Case I.) Note the hypertrophied ventricular wall in which the fibrosis is apparent. Especially note the sclerotic endocardium which lines the bulging area. Observe also the thinness of the apical myocardium where the musculature is not bulged showing that even a thin healthy wall may be as efficient as a thick fibrotic one.

the calvarium was removed, there was a gush of thin clear serous fluid in very large amounts. The dura was more than usually adherent to the calvarium and was edematous.\* The pia was tremendously edematous so that it stood up in large blebs which measured 4 cm. in diameter. Here and there, particularly over the anterior half of the cerebral lobes, the pia was scarred and clouded with fibrosis. There was no evidence of infection. The pia was also decidedly congested. The branches of the Circle of Willis, particularly the basillar, the vertebral and carotids, as well as the smaller branches, showed an exceedingly well-marked condition of arteriosclerosis with calcification. There was no distinct evidence of thrombosis. The dura of the base was generally, but not con-

siderably, thickened and more than normally adherent. There was no evidence of trauma about the head. The peripheral lymph glands were somewhat enlarged and shotty. There were no masses in the breasts. There was a slight edema of the ankles. The finger nails were cyanotic. The pupils were slightly dilated, the right being a little larger than the left. The teeth that remained were in a horrible condition and were represented merely by necrotic, movable stumps. Many of the teeth were almost completely gone superficially and their places were indicated merely by stumps.

The subcutaneous fat was very well developed. The muscles were scantily developed but of good color. The intestines seemed to occupy a normal position; the appendix was *in situ* and evidently healthy. The omentum was coiled up above the transverse colon. There were no adhesions in the pelvis; the tubes and ovaries were free. There was a tremendous congestion of the veins in the ovarian plexus. The lower border of the liver was 9 cm. below the ensiform and 6 cm. below the costal margin in the right mammillary line.

In the pericardial cavity was a slight increase of fluid which was blood-stained—about 25 c.c. There were posterolateral adhesions in the left pleural cavity and a small increase of blood-stained fluid. In the right pleural cavity there were no adhesions.

There were numerous intestinal diverticula about four feet above the cecum. There were old adhesions between the gall bladder and duodenum and between the edge of the left lobe of the liver and stomach.

The spleen was enlarged and quite firm. The capsule was only slightly and generally thickened. On section, the organ was very evidently congested and firm. The Malpighian bodies were visible.

The left adrenal was beginning to show cavitation. The right adrenal showed nothing unusual. The left kidney was somewhat smaller than usual and quite firm; the capsule stripped with comparative ease but left a generally granular surface which was torn in some few places. In the cortex there were a few retention cysts. The stellate veins were not congested. The cortex was slightly thin, the line of demarcation between cortex and medulla was faint. The glomeruli and interlobular vessels were congested. The pelvis seemed to be healthy. The right kidney resembled in all respects the left except that it was slightly larger, also there was more evident congestion in it.

The heart was quite large and the right side was moderately dilated, particularly the auricle. The epicardium was smooth. The subepicardial fat was somewhat increased in amount. On the anterior aspect of the left ventricle near the apex, was an area over which the epicardium was distinctly thickened and this area, to the fingers, felt as though the wall of the heart were thin, and almost as though the fingers could be forced through the wall. The pulmonary and tricuspid valves were healthy. There was perhaps a slight thickening of the edges of the tricuspid valve but it was not unusual. The foramen ovale was closed. The tip of the left ventricle was dilated and the area of dilatation was filled with old thrombi, some of which were softened. About this cardiac aneurism, the endocardium was exceedingly sclerotic. The columnæ carneæ which had evidently been hypertrophied, were evidently completely replaced by fibrous tissue and the dilatation had extended in such a way that some of these

fibrotic columns formed trabeculae across the aneurism. The aortic orifice was not dilated and the valves were not very much changed, although they were somewhat fibrotic. There was no evidence, however, from the appearances of an insufficiency. The valves were competent to the water test. Beginning in the transverse arch of the aorta and extending down into the iliacs, the whole vessel was the seat of an exceedingly well marked diffuse luetic mesaortitis. In the ascending part of the arch there seemed to be nothing but some patches of fatty degeneration. The coronaries showed a very well-marked patchy arteriosclerosis. In some places the vessels were almost free from sclerotic changes; in others, the tip of a fine probe could barely be passed through.

The liver was of fair size and exceedingly flabby. The lower margin of the right lobe of the liver was deformed by scar tissue and the capsule was thickened. Scattered here and there upon the surface of both lobes were small nodules measuring not more than  $\frac{1}{2}$  cm. in diameter, two or three of them raised above the surface with an area of contraction about them. Other areas of the same general appearance did not seem to be raised above the surface and had no contraction about them. In several instances there were stellate scars which extended into the liver substance. On section, the organ showed evidence of a moderate degree of passive congestion. The surface was rather deeply congested and there was evidence of a considerable amount of fatty degeneration. The whole organ was exceedingly edematous. The gall bladder was small, the walls were thickened moderately, particularly the peritoneal surface which was definitely sclerotic and in the gall bladder was an ovoid cholesterol calculus.

The right lung was of fair size, the pleura for the most part was smooth. At one point on the posterolateral inferior edge of the upper lobe was an area of almost complete consolidation over which there was a fine fibrinous exudate upon the pleura. On cross section, this area had a congested granular surface. It had a somewhat wedge-shaped form and seemed to represent a focus of acute lobular consolidation. There were apparently no thrombi in the vessels leading to it. The rest of the lung tissue was only moderately congested and scarcely edematous. The apex was scarred. The left lung showed almost a completely atelectatic and scarred lower lobe, and an almost completely normal upper lobe. The apex was irregularly scarred, irregularly contracted. On section, it was distinctly meaty, very firm and within the substance there were small areas of purulent infiltration.

The stomach was generally congested, there was something of a morocco leather appearance throughout the mucous membrane and an increase in amount of mucus on the surface between the rugae. On stretching the stomach, the rugae could be almost completely flattened. There was nothing unusual in the duodenum which was also congested.

The pancreas was distinctly congested but showed nothing else abnormal. The intestinal tract showed an increase in mucus on the surface, was somewhat edematous and somewhat, particularly in patches, congested. The contents of the intestines were bile-stained. There was a moderate degree of hyperplasia of the solitary follicles. In the large intestines, particularly in the cecum, there was a definite diphtheroid exudate upon the surface.

This extended to a lesser degree throughout the rest of the large intestines. In the sigmoid there were numbers of diverticula.

**ANATOMIC DIAGNOSIS.**—Syphilitic mesaortitis; peripheral arteriosclerosis affecting particularly the coronaries, renals, and cerebral vessels; partial cardiac aneurism; hypertrophy and dilatation of the heart; myocardial fibrosis; edema of the brain; chronic diffuse nephritis; passive congestion of the liver, kidney, spleen and pancreas; acute lobular pneumonia; pulmonary atelactasis; chronic catarrhal gastritis; chronic follicular enteritis; acute diphtheroid colitis; pediculosis; scoliosis; cholelithiasis.

**REMARKS.**—In this case the basis of the myocardial changes seemed to rest upon a luetic process which had produced very definite coronary changes together with myocardial fibrosis. This latter may have been due either to the infection, or to the coronary condition, or, more probably, to both. There were



Fig. 2.—Case A-4464. (Case II.) In this case the heart is apparently elongated and immediately at the apex is the irregular slit indicating the point of rupture of the aneurismal sac which produced the appearance of increased length.

no pericardial adhesions, but the endocardium and epicardium were hyperplastic, conditions which prevented rupture of the aneurism. Evidently the thrombi within the sac were not essential in offering resistance to rupture, for they were softened. Death in this case was evidently directly associated with the lobular pneumonia, which, with the acute pseudomembranous colitis, may be looked upon as evidences of a terminal infection.

#### CASE II.

E. T., No. A-4464, was brought to the Receiving Ward of the Cincinnati General Hospital, as an emergency case, on June 30, 1916. He died within a few minutes after arriving at the hospital. No clinical history was obtained.

**AUTOPSY PROTOCOL.** (M., J. S.)—The body was that of a well developed, well preserved, white man about 50 years of age. Postmortem rigidity was exceed-



ingly well marked and lividity was present in the dependent portions. The pupils were equal and dilated. The teeth were discolored though in fair condition, and the gums were pyorrheic. The lips were cyanotic. The chest was barrel-shaped, the abdomen somewhat pendulous, and the superficial veins were slightly distended. Both ankles seemed enlarged, and the right seemed to have undergone some bony ankylosis, as it could not be moved.

The subcutaneous fat was abundant and the superficial muscles were well developed. Upon removing the sternum, the lungs partially collapsed. There were about 500 c.c. of a slightly blood-tinged fluid in each pleural cavity. There were no pleural adhesions in the left cavity. From the right anterior axillary line to the vertebral border and as far upward as the third rib, the right lung was adherent by fibrous adhesions. The mediastinal and pericardial fat was very abundant. When the pericardial sac was opened, a large quantity of blood-tinged fluid and clot flowed out. The amount of blood in the pericardial cavity was about 20 ounces. The omentum was filled with fat and the mesentery similarly contained a great amount of fat. Around the cecum there were numerous old fibrous adhesions and the appendix was adherent behind the ileum and was represented by a short fibrous tag containing no lumen. There was no fluid in the abdominal cavity. There were no adhesions about the gall bladder.

The left lung was voluminous and crepitated throughout. There were a few small shotty areas beneath the pleura which no section represented obsolescent calcified tubercles. The cut surface of the lung showed no gross abnormality. The right lung contained the remains of numerous fibrous pleuritic tags. The lower lobe was soft, and friable, but crepitated throughout. This lobe was considerably congested and edematous and was the only portion of the lung that showed any pathologic change.

The heart was about twice its normal size. There were numerous old fibrinous adhesions between the epicardium and the pericardium. These adhesions were very short ( $\frac{1}{8}$  inch) and drew the myocardium out into a small aneurism. The apex of the left ventricle had ruptured; the site of rupture occurring in a thin aneurismal sac measuring about 2 inches in diameter, the walls of the sac being represented evidently by fibrous tissue lined by endocardium throughout. Over the epicardium covering the sac, were numerous blood clots undergoing organization and the endothelial lining of these contained a number of small fibrinous vegetations. The heart and aorta were not opened but were sent to the museum intact.

The liver was about of normal size; it had an irregularly granular surface of a pinkish-blue color. It cut with increased resistance and the cut surface was purplish-red in color, and showed an increase in both fibrous and fatty tissue; the lobules stood out prominently, due to fibrous contraction. The gall bladder contained about twelve black, irregularly shaped concretions, all of about the same size. The concretions were rather soft. There was present in the gall bladder about 1 ounce of greenish-black fluid.

The kidneys were slightly enlarged. The capsules stripped with some difficulty leaving a mottled surface on which the stellate veins appeared moderately congested. The cortex was diminished in thickness and the line of demarcation between cortex and medulla was lost. The glomeruli were visible as red con-

gested points. The interlobular veins were congested. The renal pelvic fat was greatly increased.

The spleen was greatly enlarged. Upon the upper surface there was a large cyst containing about 500 c.c. of a clear straw-colored fluid. The inner lining of the cyst was thin, pale and glistening and was thrown into folds which readily disappeared on stretching. The cyst did not extend into the substance of the spleen, but lay within the capsule. The cut surface was soft and pulpy and very friable.

ANATOMIC DIAGNOSIS.—Ruptured cardiac aneurism; cyst of the spleen; chronic diffuse nephritis (interstitial type); acute splenic tumor; cirrhosis of the liver; absollescent pulmonary tuberculosis.

REMARKS.—This case illustrates again the abrupt termination in cases of cardiac aneurisms when the dilated portion of the myocardium is not protected by ventricular thrombi or pericardial adhesions. It also illustrates a condition of affairs which has given rise to much discussion, namely, one in which there are a few pericardial adhesions which it has been suggested have been an important factor in the production of dilatation by exerting traction on the cardiac wall. Diverticula of other organs,—esophagus and intestines,—are sometimes caused in this way, hence the term "traction diverticula." Whether or not traction was an important factor in this case cannot be stated, though the facts suggest it.

#### CASE III.

B. F., No. 3708, was admitted to the Cincinnati General Hospital on July 29, 1915. He died on November 4, 1915. The clinical history has been lost.

AUTOPSY PROTOCOL.—The body was that of an old gray-haired man, 5 feet tall. It was evidently, though not markedly, bowed forward. It was, as to the upper part, rather illy nourished; as to the lower, well supplied with fatty tissue. Rigor mortis was incomplete. Lividity was brilliant. To the left of the coccyx was a superficial bed sore 6x4 cm. in diameter. Five centimeters below the left great trochanter was a second bed sore, round, punched-out in appearance, its base on the muscle and its sides sharp and clean. It measured 4 cm. in diameter. The left leg, commencing 17 cm. below the great trochanter, was greenish, a hue that increased as the foot was approached, until in the foot itself, it was a blackish green and showed evidence of liquefaction necrosis. The terminal half of the foot had been amputated and at the site of the operation, the necrotic metatarsal bones protruded from the surrounding foul greenish dead tissues. The right leg had suffered not at all though the right foot in the region covering the os calcis laterally and on the plantar surface, was gangrenous. Just below the external malleolus was a deep penetrating ulcer 6x2 cm. Under the internal malleolus was a second, round one 2.5 cm. in diameter.

The interesting feature of this case was the cardiovascular system. The heart was enlarged and dilated especially on the right side, but also considerably on the left. The muscle was brown. Upon the anterior surface of the left ventricle near the base medially, was an area which to the touch suggested the presence of bone in the myocardium. Scattered about this, were numerous myocardial scars showing as pale striated areas beneath the epicardium. The

left coronary was exceedingly firm and very sclerotic. It was so thick and impregnated with lime salts that it was opened with difficulty. The left branch of the descending branch of the left coronary was also exceedingly sclerotic and almost obliterated. It was this branch which led directly to the firm area mentioned above. When the ventricle was opened, it appeared at once that the area of hardening, which measured some  $3\frac{1}{2}$  cm. in diameter, was actually a very thin lamella of fibrotic myocardium not more than 1 mm. in thickness and densely filled with lime salts. The endocardium of both ventricles was fibrotic and the columnæ carneæ and papillary tendons were also sclerotic. The valves were not especially damaged though they were slightly fibrotic. The aorta also was not remarkably affected. At but one point in the arch was there a well marked area of atheroma, though there were a number of fatty areas of degeneration in the intima. The main branches of the aorta were, however, more affected by the sclerotic process, and this was especially true of the main branches of the subclavians, and of the iliacs. The spermatics, internal epigastrics, obturators, profundas and the femorals, popliteals, and their terminal branches all simulated calcified tracheas, and this was especially noticeable in the left leg. Here, the popliteal was almost completely obliterated just above the origin of the anterior tibia.

The lungs were very healthy in general appearance, although the left was almost completely adherent to the mediastinal tissues and to the parietes. Nevertheless on section they were pale and dry and had no areas of consolidation.

The liver weighed 1175 grams, and was pale brownish yellow, nodulated and sclerotic. The bile ducts were patent.

The spleen (120 grams) was flabby, the edges rounded, the capsule thickened at scattered points, and the pulp slightly fibrous, showing enlarged Malpighian corpuscles.

The pancreas, aside from a very definite fatty infiltration, showed no microscopic changes.

The kidneys (left 120, right 90 grams) were small, granular and pale. Each showed the remains of fetal lobulation. To each the capsule was moderately adherent. The cortices were thin and gray, the medullæ fibrous.

The adrenals showed increase of medullary tissue with focal accumulations of cortical tissue simulating adenomas. The pelves of the kidneys and the ureters were healthy. The bladder was contracted and held an ounce or so of a cloudy urinous fluid. The mucous membrane was intensely reddened, and thickened. Upon a number of the folds were patches of fibrin forming white points. The urethra was not inflamed. The prostate was small and fibrotic. Both testicles were very small, scarcely larger than ordinary ripe olives.

The brain was exceedingly edematous. There were patches of arteriosclerosis in the arteries of the base, but no evidence of blocking of the lumens.

**ANATOMIC DIAGNOSIS.**—Diffuse peripheral arteriosclerosis; coronary arteriosclerosis; gangrene of right leg, and left foot (partial); partial cardiac aneurism; atrophic cirrhosis of the liver; myocardial fibrosis; cardiac hypertrophy and dilatation; fatty infiltration of the pancreas; edema of the brain; arteriosclerotic kidneys; acute catarrhal cystitis; atrophy of the testicles.

**REMARKS.**—This case illustrates a relatively rare location of a cardiac

aneurism, and it also illustrates typically what may happen to a section of myocardium whose blood supply is so gradually decreased that the functional tissue is completely replaced by fibrous tissue, and in which the fibrous tissue later becomes infiltrated with lime salts.

In this case the changes in all the organs are predominantly sclerotic which indicate the presence of very general vascular changes. The slow progress of this condition is indicated in the age of the patient.

#### CASE IV.

C. G., No. 6136, was admitted to the Cincinnati General Hospital on November 24, 1915. He died on December 22, 1915.

CLINICAL NOTES.—The patient came to the hospital complaining of short-



Fig. 3.—Case 6136. (Case IV.) Note the lamellated clot more than half filling the left ventricle, and also note the extreme fibrosis of the ventricular myocardium. At the tip of the ventricle the clots fill the meshes of the fibrotic myocardial wall.

ness of breath. He had previously been in the hospital from October 15, 1915, to October 27, 1915.

For a year he had felt under the weather. He had been a heavy drinker of spirits, but denied venereal infection. The difficulty of which he complained troubled him for at least a year, during which time he was especially uncomfortable at night. For three months he reacted badly to the slightest exertion. With this he had a dry cough. Two weeks before his first admission he spat up a little frothy, pale red, bloody fluid. For six months he had some edema of the feet and legs, and for some months had urinated frequently. For two weeks he had an edema of the external genitalia. His bowels were regular.

On October 17, the following note was made: Pupils equal; right radial pulse stronger than the left which is almost imperceptible. Apex beat visible and palpable to the left of the nipple in the fifth interspace; impulse strong and heaving. The first sound at the apex is reduplicated; the second pulmonic

slightly accentuated. There are no audible murmurs. There was visible pulsation in the first interspace and well marked retrosternal dullness. There was no tracheal tug. The legs below the knees were moderately edematous.

Relative cardiac dullness:

First interspace	2.3 cm. to right.
	2.5 " " left.
Second interspace	2.8 cm. to right.
	3.3 " " left.
Blood pressure: Right arm—	systolic 156, diastolic 125.
Left " — " "	144, " 120.

The radiographic report on October 20th was as follows: "The heart is lying in a transverse position. Its long diameter has apparently increased. Upper mediastinal shadow is broader than usual and from the relation of its curves suggests an enlargement of the aortic arch." A fluoroscopic examination of the chest revealed no circumscribed enlargement of the aortic arch, but instead, a diffuse enlargement suggesting a dilatation of the aorta.

The Wassermann reaction was negative.

During his stay in the hospital his temperature remained normal except for an occasional rise to 99.5° (maximum). Pulses remained between 60 and 100; respiration, 20—30.

He was discharged, unimproved, at his own request on October 27, 1915.

At the time of his second admission on November 24, 1915, the patient was generally edematous, and exhibited Cheyne-Stokes breathing. His urine contained albumin and casts. Over the base of each lung there were signs of edema. The heart was enlarged; 15 cm. to the left; 3 cm. to the right. The cardiac rhythm was irregular. A faint systolic bruit was heard just below the left nipple.

As time went on there was no improvement. The patient was drowsy most of the time; Cheyne-Stokes breathing persisted; the pulse became gradually weaker, and death occurred on December 22.

CLINICAL DIAGNOSIS.—Dilatation of the aorta; arteriosclerosis; chronic diffuse nephritis; myocardial insufficiency; mitral insufficiency.

AUTOPSY PROTOCOL (G.)—The body was that of a well developed, poorly nourished negro, apparently about 50 years old, and six feet tall. The extremities were edematous. The teeth were in exceedingly poor condition, those that were present,—two above and seven below. The right pupil was twice the size of the left.

When the chest was opened the lungs did not collapse; each pleura contained about 500 c.c. of clear fluid, and no adhesions. Both lungs were congested and edematous. The left showed an apical scar. In the right lung, in the lower lobe was an area of consolidation about the size of a hen's egg.

The pericardial fluid was not increased. The heart was markedly enlarged and also dilated, the latter condition being especially prominent in the right ventricle. The tricuspid valves were thickened and fibrotic. The left ventricle was exceedingly hypertrophied, and near the apex was a mass of thrombus attached to the myocardium which was extremely thin and fibroid. The myocardium was, generally, the seat of a well marked fibrosis. The aortic valves were

slightly thickened, and the aorta showed, especially about the mouths of the coronaries, thickening and many scattered patches of atheroma. Subintimal fatty changes were especially prominent. The whole aorta, but particularly the arch, was somewhat, though not strikingly dilated. The mitral valve was sclerotic, and the leaflets much thickened.

The peritoneal cavity contained a small amount of free fluid. The mesenteric glands were enlarged and firm, but showed no visible evidences of disease. The stomach, liver and spleen were congested. The liver was of normal size but unusually firm. The pancreas, intestines and adrenals were not unusual. The ureters, bladder and prostate showed nothing of note.

The kidneys were small and scarred as if by healed infarcts. The capsules stripped readily leaving somewhat granular surfaces. The cortices were narrowed, and there was some evidence of sclerosis.

Permission to remove the brain was not given.

**ANATOMIC DIAGNOSIS.**—Chronic diffuse nephritis; myocardial fibrosis; cardiac thrombosis; cardiac hypertrophy and dilatation; aortic dilatation; aortic atherosclerosis; terminal lobular pneumonia; congestion of lungs, liver, spleen and stomach; anasarca.

**REMARKS.**—This case offers an exceedingly good illustration of what happens in preparation for, and prevention of, cardiac aneurisms. The whole myocardium was the seat of an extensive very diffuse fibrosis, which had been accentuated near the apex of the left ventricle. At that place the wall of the cavity was composed almost entirely of fibrous tissue, and was, compared with the rest of the myocardium, very thin. Evidently there had been the tendency for the heart to bulge out at this point. But during the period of incipient bulging, thrombosis had occurred, and over the whole area of thinning, clots had been laid down forming lamellæ which had been sufficiently firm to protect the ventricular wall. It seems reasonable to expect that, had the myocardium as a whole been more vigorous; had there been a higher blood pressure, and, therefore, a more rapid stream of blood, a more distinct bulging would have been produced.

#### CASE V.

**CLINICAL NOTES.**—M. G., hospital No. A-6479, a colored female, aged 35, married, a washwoman, was admitted to the Cincinnati General Hospital, September 30th, 1916, complaining of "shortness of breath, and swelling of the legs and stomach."

**Family History.**—The patient's mother died of dropsy at the age of 55 years.

**Past History.**—The patient had "measles," "mumps" and "whooping cough" in childhood. She had arthritis of both ankles, with swelling and pain, after she was grown. She denied having had venereal infections. The patient said she used to drink about three glasses of beer daily but no whiskey until six months ago. She did not use tobacco, or drugs. She had two children, aged 18 and 5 years, both living and well. The patient was in the hospital last Spring and at that time she was emaciated. She complained of pain in the epigastrium. There was a tumor just to the left of the median line and above the navel. This was

about the size of a pigeon's egg, or larger. It had a pulsation which at times seemed to be expansile although the tumor and pain disappeared on the administration of iodide potassium in large doses and the patient was discharged after a few weeks, feeling well.

*Present Illness.*—The patient has been feeling badly ever since the first of September. She had had shortness of breath on the least exertion. She frequently has had to sit up at night because of dyspnea. About a week after the onset her feet began to swell. The swelling has gradually increased and the abdomen began to swell before she came to the hospital.

*Physical Examination.*—The patient's general condition was fair. The pu-

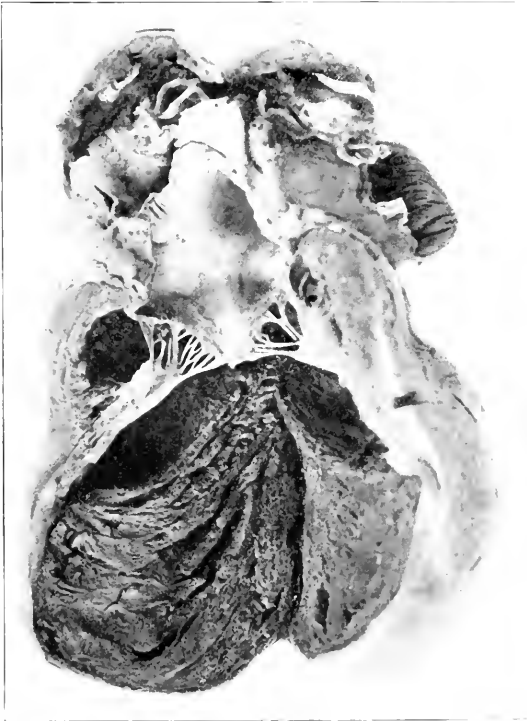


Fig. 4.—Case A-6479. (Case V.) In this case the heart was elongated and the tip bulbous. The aneurismal sac was well developed and filled with a recent red clot in which organization was commencing about the periphery.

pils were moderately dilated. They reacted to light and accommodation. There was no nystagmus. The conjunctivæ were slightly injected. The ears and nose were negative. The tongue was moist. Many teeth were missing. Those which remained were in poor condition. There was pyorrhea alveolaris. There was a general glandular enlargement, including the epitrochlears. The glands were only slightly enlarged and discrete. The thorax was symmetrical and expansion was equal. The lungs were negative on percussion and auscultation, except for slight dullness at the bases, without alteration in the signs on auscultation. The heart apex was one inch outside the mid-clavicular line. The cardiac dullness was not given. The rhythm was irregular. There was a systolic murmur

at the apex which was also heard along the sternum with a diastolic murmur at the aortic area. The abdomen was distended. There was a movable dullness in the flanks. There was moderate edema in the lower extremities. The reflexes were normal.

A Wassermann reaction was positive. The sputum was examined for tubercle bacilli, and none were found. The urine was yellow. Specific gravity 1.010, slightly acid, albumin present. There was no sugar. Microscopically granular and hyaline casts and leucocytes were found.

On October 10th, the patient had improved very much. The edema of the lower extremities had practically disappeared and the dyspnea was less marked. On the 19th, she had continued to improve. The edema had disappeared and she was allowed to be up for an hour. On October 25th, the patient awakened suddenly about 5 o'clock in the morning complaining of a very severe, sharp, continuous pain in her abdomen. She was found in a sitting posture and had both hands on the abdomen. She was also vomiting at times. On examination there was some distension of the abdomen but no apparent localized tenderness although she complained of pain in the right upper quadrant. There was no rigidity and no masses palpable in the abdomen. The following day she still complained of pain in the abdomen. There was rigidity in the upper right quadrant. The liver was enlarged a finger breadth below the costal margin. On November 9th, the apex impulse was diffuse, most marked in the sixth interspace, 7 inches from the mid line. Relative cardiac dullness extended 8 inches to the left in the seventh, 7 inches in the sixth, and  $6\frac{1}{2}$  inches in the fifth. The dullness to the right was not given. In the seventh interspace retraction was noticed during each systole. No murmurs were heard at the apex. There was a faint diastolic and a blowing systolic murmur at the base. Traubes' space was dull. The liver was 11 cm. below the xiphoid cartilage and 5 cm. below the costal margin in the nipple line. On November 12th, a catheterized specimen of urine showed 2 grams of albumin per liter with numerous hyaline and some cellular casts, also granular casts and pus cells. Moist rales were heard at the base of each lung. November 13th—aspiration of the pericardium yielded 6 ounces of bloody viscid fluid.

X-ray examination of the gastrointestinal tract on October 10th, was entirely negative.

On November 8th, there was marked increase in the transverse diameter of the heart, especially involving the left ventricle. Fluoroscopic examination of the chest showed the transverse diameter of the heart markedly increased in width, the right side of which does not pulsate.

The patient's temperature was for the most part normal or subnormal with occasional rises to  $99^{\circ}$  or  $99.6^{\circ}$ . The pulse was between 80 and 100, respiration 120 two days before death. Respirations were between 20 and 30.

CLINICAL DIAGNOSIS.—Nephritis; cardiac hypertrophy and dilatation; aortic insufficiency; pericardial effusion; passive congestion of the liver; ascites, general anasarca; passive congestion of posterior lower lobes of the lung; uremia; syphilis; hemopericardium.

AUTOPSY PROTOCOL (G. G.)—*Necropsy*.—The body was that of a well



nourished negress about the age of 35. Postmortem rigidity was very slight and there was slight lividity in the dependent portions. Over the left hip was a linear scar which ran horizontally and was about 3 inches long. There was a small trochar wound about 1 inch to the left of the midsternal line and in the fourth interspace. The teeth were in poor condition, the majority were present but many were in a carious condition. The eyes were equal; the pupils were not dilated but there was present an internal squint of the right eye. The lower extremities on both sides showed some edema. The subcutaneous tissues were very moist and edematous over the whole abdomen and chest. The musculature of the abdomen was very poorly developed. There was an area of interstitial hemorrhage just beneath the trochar wound about 3 inches long and  $2\frac{1}{2}$  inches wide. When the abdominal cavity was opened it was found to be filled with a straw-colored fluid. The stomach was distended but the small intestines were contracted and the bladder was about empty. The peritoneum was very much injected and both the lesser and greater omentum were deeply injected. The appendix was *in situ* and apparently healthy. There were many adhesions between the anterior surface of the right and left lobes of the liver and the abdominal wall. The left pleural cavity was fairly free from adhesions but there was present about 4 ounces of a bloody fluid. When the chest was opened, the left lung did not collapse but the right did. On the surface of the pericardium beneath the point of the trochar wound was another interstitial hemorrhage of about 3 inches in diameter. The pericardium was filled with about 8 ounces of a bloody fluid. The pericardial cavity contained numerous adhesions mostly about the apex.

The ascending colon was firmly fixed to the right kidney. Both kidneys were strongly adherent to the posterior abdominal wall and were with difficulty removed. The right was found adherent to the ascending colon and also to the posterior wall. Extending down into the iliac fossa there was found a tissue that resembled to some extent adrenal tissue and which infiltrated all the interstitial tissues of the groin and to some extent was found within the anterior abdominal wall. This was true of both sides but was more extensive on the right than on the left. The stomach was injected and there was a slight excess of mucous material on the surface. The rugæ were not very apparent and the mucosa of the stomach presented the appearance of morocco leather. Otherwise the organ was normal. The duodenum was quite intensely injected and there were several small hemorrhages in the lower part of the duodenum. There was nothing more than edema and congestion of the gastrointestinal tract. The heart measured 18 cm. from the root of the aorta to the apex, and had a generally blunt cylindrical form. The whole apical region was somewhat bulbous and was separated to a certain extent from the rest of the heart by a slight groove about 6 cm. above the apex. This bulbous apical region had a semifluctuating feel which suggested the presence of an aneurism at the apex. The whole pericardium was roughened and covered with the tags of old adhesions in which there were occasional small areas of hemorrhage. At one point just about the middle of the surface of the right auricle was a fine puncture wound surrounded by an areola of extrava-

sated blood which measured about 3 cm. in diameter. This puncture wound apparently extended completely through the myocardium into the cavity of the right ventricle where it impinged upon a small mass of mural thrombi. The right auricle was very considerably dilated and in the auricular appendage there were a few old mural thrombi. There was a certain amount of jaundiced appearance in the mural endocardium, an appearance which was also seen to a somewhat less extent in the vessels. The right ventricle was relatively contracted. The endocardium along its left posterior side was fibrotic and milky and the papillary muscle of the left tricuspid leaflet were fibrotic. In the tip of the right ventricle there were masses of old mural thrombi. The left auricle was somewhat dilated but aside from hemolytic pigmentation or staining showed no unusual appearances. The mitral valve was evidently not dilated. The left ventricle was tremendously dilated but was filled almost completely with a laminated clot which was rather firm peripherally but quite soft centrally. This clot reached almost to the aortic valves. Apically the myocardium was replaced by a thin zone of connective tissue from two to three mm. thick. Nearer the base of the heart the myocardium became about 15 mm. thick and showed some evidence of hypertrophy. The mitral papillary muscles and the ventricular myocardium were distinctly fibrotic. The mitral valve leaflets showed no changes on the margins and the chordæ tendineæ were not contracted. The aortic leaflets were apparently healthy and the orifice was sufficient by the water test. The aorta itself showed some slight patches of subintimal hyperplasia and some slight puckering of the intima. These changes were shown particularly in the transverse arch just in the neighborhood of the great vessels. Were it not for the hemolytic staining, these patches would be typical luetic ones. Aside from that, the aorta showed no changes. There were no dilatations and no sclerosis. The peribronchial glands showed some calcification. The coronaries were, in their basilar segments at least, thin and normal in appearance. A fine probe could be passed however but 5 cm. down the descending branch of the left coronary at which point this vessel seemed to be sclerotic. Sections from this part of the coronary were saved for examination. Below this point the pericardium seemed to be far more thickened than about the base and the path of the vessel could not be distinguished in the hyaline connective tissue.

The left lung had a rather firm feel and its outer surface was dark gray in color. The cut surface of the lung showed an intense congestion and edema, more intense to the center and decreasing towards the periphery. The right lung was more contracted than the left, had a distinct meaty feel and did not crepitate in any portion. On the inner border of the middle lobe was found a dark consolidated area which on section proved to be an infarct. This infarct measured about  $2\frac{1}{2}$  inches from the border to the apex of the triangle and about 2 inches high along the border of the lung. The cut portions of the lung showed only edema and congestion. The upper lobe of the right lung was fairly healthy and showed very slight if any congestion.

The liver showed a dirty brown grayish appearance, its edges were sharp and irregular and the whole organ was decreased in size and very flabby. Over

the surface were found many areas of a yellowish color and many fibrous tags. The organ cut rather firm and on section there were found the typical patches of passive congestion with areas of fatty degeneration. The cut surface of the organ was of a yellowish-reddish brown color. The gall bladder was filled with a brownish, almost clear mucous fluid in which there were found no stones nor any gravel.

The spleen was hard, contracted and had a very fibrous feel. The pulp was of a deep red and there was an evident increase in fibrous tissue.

The right kidney was firm and generally pale. The capsule was quite firmly adherent by evidently recent fibrous and some fibrinous adhesions. When the capsule was removed, it was seen that the capsule was massively filled with hemorrhagic patches some of them petechial, most of them larger and confluent. Not only was the capsule involved in this process but also the perirenal tissues which had a distinctly indurated feel and in which the fat at certain points had a peculiar orange appearance which suggested congested thymus tissue. The surface of the kidney was generally yellow with massive and small petechial hemorrhages scattered generally over the surface. On section, the kidney appeared to be completely infarcted, and section of the renal artery showed a mass of thrombotic material almost completely blocking the vessel. The whole cut surface of the kidney was jaundiced and in the regions where the infarction had not been complete the substance was distinctly pinkish-gray. The left kidney was about the same size as the right. The capsule removed with fair ease leaving a very finely, indistinctly granular surface upon which the fetal lobulations were very distinct. The whole organ was rather firm, decidedly edematous, the stellate veins were not congested. On section, it appeared that the cortex was increased in thickness, there was some evidence of a moderate sclerosis in the pyramids, the line of demarkation between cortex and medulla was fairly distinct and showed some evidence of fibrosis and was distinctly jaundiced; the glomeruli could be seen fairly well as congested points and the interlobular vessels were injected. There was no evidence of thrombosis or infarction in this organ. The blood vessels, especially the arteries, were deeply jaundiced.

**ANATOMIC DIAGNOSIS.**—Aneurism and thrombi of the left ventricle; fibrosis of the left auricular walls, mitral valve and papillary muscles of the left ventricle; trochar puncture wound of the heart; congestion and edema of all the viscera; pulmonary infarct; complete infarction of the right kidney; acute diffuse nephritis of the left; chronic passive congestion of the liver.

**REMARKS.**—This case represents the type in which a moderately large area of the ventricular muscle was damaged, and in which the intracardiac pressure was not excessive. The result was, therefore, that a very considerable gradual dilatation occurred. No thrombi were formed in this aneurism until a short time before death and then the sac was rapidly filled with clot which reached almost to the valves. From this thrombotic mass, a large bit was carried to the kidney where it completely blocked the renal artery.

## SUMMARY.

The series of cases reported in this paper has to do with the formation of cardiac aneurisms. Cardiac aneurisms and aortic aneurisms resemble one another in every aspect so far as pathogenesis is concerned and differ only in the fact that they appear in different organs.

One of the cases reported showed the beneficial result of thrombosis in a heart which otherwise might have developed an aneurism (6136); one showed an early stage of aneurism formation near the apex (A-2775); one showed an early stage of aneurism formation in an unusual situation, and also illustrated calcification of the fibrous tissue forming the wall of the sac (3708); one showed a rupture of a chronic aneurism in spite of a few pericardial adhesions; and one showed a fully developed large chronic aneurism (A-6479).

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## A STUDY OF THE OCCURRENCE OF IMMATURE POLYNUCLEAR LEUCOCYTES IN THE CIRCULATING BLOOD IN PULMONARY TUBERCULOSIS AND OTHER INFECTIOUS DISEASES\*

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AMONG the many investigations of the activities of the polymorphonuclear leucocyte, those of greatest interest to the clinician have dealt with the relation of the leucocyte to the common infections. In the study of clinical cases, the estimation of the leucocyte count and the examination of the stained blood film have long been routine procedures. More recently variations in the morphology of the polynuclear leucocytes in the blood stream have been recognized as occurring in certain diseases, and attempts have been made to determine the relation of these changes to the clinical condition.

Arneth, in 1905, classified the polynuclear leucocytes of the circulating blood according to the number of lobulations of their nuclei, and noted that in infectious diseases, especially tuberculosis, the proportion of the cells of Classes I and II, those with single or two lobed nuclei, was increased. This he interpreted to mean that the younger, immature leucocytes were relatively more numerous than in the normal blood. For example, the following represent respectively a normal count, and one which might occur in such an infectious disease as tuberculosis:

	CLASS I.	CLASS II.	CLASS III.	CLASS IV.	CLASS V.
Normal:	12	35	30	18	5
Abnormal:	52	37	10	1	0

\*I am indebted to Dr. W. P. Buffum, Jr., and Mr. John von der Leith for aid in making some of the blood examinations.

A count like the second of these, Arneth calls "a shift to the left," a shift of the majority to Classes I and II.

It was noted by Arneth that in active pulmonary tuberculosis, although leucocytosis was absent, a well marked "shift to the left" usually occurred. He supposed that the relatively immature cells of Classes I and II were less able to cope with an infection, and that therefore the resistance of a patient who showed such a "shift" must be lowered and the prognosis must be poor.

Arneth's classification has been widely used by many investigators, especially in the study of pulmonary tuberculosis. Opinions of its value vary greatly, and on reading the literature one is led to suspect that its true significance is not appreciated. P. H. Ringer, for example, says "It must be distinctly understood that the Arneth blood picture can in no way be taken to indicate the amount of lung involvement or the stage of the disease. It is simply and solely an index of the resisting power of the patient." R. S. Cummings regards it of no diagnostic worth, and of value in prognosis in doubtful cases only. The opinion of Solis-Cohen and Strickler is that "Few that take up the study of the picture carefully ever abandon it entirely, but he who places implicit confidence in its readings and bases his prognostic opinion chiefly thereon will not infrequently be led into error." More unfavorable is the statement of Bushnell and Treuholtz that "In advanced and active tuberculosis the method of Arneth gives us as a rule no information which we do not already possess."

In view of the essentially vague opinions as to the significance and value of the Arneth method, it seemed possible to the writer that a study of the leucocyte picture in pulmonary tuberculosis and other infections, and a careful correlation of this picture in the cases studied to other clinical and pathological facts, might yield information of interest. Therefore, counts were made on a number of patients with pulmonary tuberculosis and on cases of other infectious diseases as they were encountered in the routine of clinical work, and so far as possible the nature and degree of activity of the lesion was determined. In a few cases of fatal pulmonary tuberculosis a postmortem study of the bone marrow was carried out.

It became apparent at once in making leucocyte classification that the personal element must be a factor which causes great variation in results. Ringer states that he counts as one, two nuclear lobes connected by an isthmus of nuclear material "a third or more the thickness of the nucleus," and as two, lobes connected by a narrower strand. He also counts superimposed nuclei as separate units. Certain other observers insist that to be counted as separate, lobes must have only a minute thread-like strand connecting them, and that when nuclei are superimposed they must be counted as one, or else the cell must be left unclassified. It has seemed to the writer that the latter method allowed of far less personal variation in making the estimations, and it has therefore been followed. All doubtful cells have been disregarded. In the results herewith presented it will be noted that the normal cases show a larger proportion of cells in Classes I and II than do the normal counts published by many other observers; but it is probable that this can be explained as due entirely to the personal interpretation of the rules used in classifying the cells. Ordinary

CASE No.	DISEASE	WHITE COUNT	ARNETH COUNT					RATIO	REMARKS AS TO CLINICAL CONDITION, TEMPERATURE, ETC.
			I	II	III	IV	V		
1	Normal.....		29	40	29	2	0	0.4	Normal.
2	".....		35	40	10	4	0	0.65	"
3	".....		28	45	15	1	0	0.46	"
4	".....		17	31	32	10	0	0.23	"
5	".....	8,200	28	42	25	5	1	0.38	"
6	".....	6,000	30	32	22	2	0	0.54	"
7	".....		4	27	5	1	0	0.12	"
8	".....	9,000	38	43	15	4	0	0.61	"
9	".....	9,500	59	72	14	0	0	0.68	"
Av'ge of normal cases....			30	41	19	2	0.1	0.48	
10	Pericarditis and nephritis	17,900	29	27	29	4	1	0.4	Temperature 100 to 101. Recovery in a few weeks.
11	Endocarditis (post-rheumatic).....	11,000	45	36	12	5	1	0.83	T. 99.5 to 100 for several weeks.
12	Alveolar abscess (small).....	14,600	24	52	18	6	0	0.31	T. 99. General malaise and aches.
13	Acute appendicitis.....	20,000	66	91	37	5	1	0.49	T. 100. Operation. Inflamed appendix. No pus.
14	General myositis..... (Chronic).		45	35	14	5	1	0.81	T. 100. Gradual recovery.
15	Bronchiectasis..... (Improving).		50	54	19	4	0	0.65	T. normal. Feels well. Sputum slight.
16	Lobar pneumonia.....	28,000	124	26	3	0	0	4.2	Acute lobar pneumonia with recovery.
17	Mar. 3. pulmonary embolus c. sept. pneu.....	31,000	73	19	7	1	0	2.7	Embolism following appendectomy. Markedly toxic.
	Mar. 8. pulmonary abscess.....	16,000	84	12	4	0	0	5.2	Coughed up large quantities of foul pus.
	Mar. 12. pulmonary abscess with gangrene....	12,000	93	8	0	0	0	12.3	T. septic in type 100-103. Large quantities of sputum. Oper'n 2 days later.
	Mar. 26.....	14,500	88	10	2	0	0	7.2	T. 98.6-100. Draining fair amount pus.
	April 22.....	14,000	45	35	17	4	0	0.8	T. normal. Wound not draining.
18	Bronchiectasis.....		108	16	5	0	0	5.1	Large quantities of purulent sputum.
19	Bronchiectasis..... (Question of T. B.)		83	66	7	2	0	1.1	Considerable purulent sputum.
20	Empyema.....	25,000	88	39	8	0	0	1.9	T. 100. Draining some pus from operation wound. Looks sick.
21	Empyema.....	19,000	68	31	1	0	0	2.1	T. 100 for 10 days.
22	Appendicitis.....	12,900	63	28	7	2	0	1.7	Operation 2 days later. Appendix apparently continuously discharging pus into cecum.
23	Carcinoma (abdominal).....	25,000	104	47	14	2	0	1.6	Operation. Death. Specimen shows no necrosis. T. 100.5. Pulse 120, weak.
24	Mar. 16. Appendicitis. 9 days post operative....	29,400	54	32	11	3	0	1.2	Wound draining. Temperature 100.4.
	Mar. 25. Improvement.....	14,600	48	38	9	5	0	0.9	Wound no longer draining.
	Mar. 31. Improvement.....	14,000	22	23	5	0	0	0.8	Wound no longer draining.
25	Malaria.....		63	36	1	0	0	1.2	
26	".....		73	24	3	0	0	2.7	
	".....	9,600	67	31	2	0	0	2.0	

CASE No.	DISEASE	WHITE COUNT	ARNETH COUNT					RATIO	REMARKS AS TO CLINICAL CONDITION, TEMPERATURE, ETC.
			I	II	III	IV	V		
27	Pulmonary tuberculosis	.....	33	33	23	6	0	0.5	Arrested. T. normal. No sputum.
28	" "	.....	35	40	27	13	1	0.4	Chronic, very slowly progressive type. T. 97-99. Considerable sputum.
	Dec. 16, '14	.....	85	57	16	2	0	1.1	Chronic, very slowly progressive.
29	" "	.....	61	29	9	1	0	1.6	Chronic, very slowly progressive.
30	Mar. 16, '15	.....	82	33	5	1	0	2.1	Considerable sputum.
	Nov. 14, '14	.....	88	29	12	1	0	2.1	Chronic, very slowly progressive.
31	Mar. 16, '15	.....	77	36	6	0	0	1.8	Considerable sputum.
	Jan. 12, '15	7,000	102	30	9	1	0	2.5	Chronic, very slowly progressive.
32	Mar. 16, '15	.....	58	55	20	2	0	0.7	Sputum slight.
	Dec. 14, '14	.....	44	42	17	1	0	0.7	T. normal. No sputum.
33	Mar. 15, '15	.....	84	30	15	0	0	1.9	Slight activity.
	Dec., '14	.....	26	33	10	3	0	0.5	Chronic, slowly progressive. T. usually normal.
34	Jan., '15	.....	70	50	26	4	0	0.9	Sputum thick: 50 to 80 c.c. daily.
	Mar., '15	.....	115	32	4	0	0	3.2	Sputum thick.
35	Jan., '15	.....	83	47	14	1	0	1.3	Chronic, very slowly progressive.
	Mar., '15	.....	94	44	4	0	0	1.9	Chronic, very slowly progressive.
36	Nov., '14	.....	49	34	21	0	0	0.9	Slowly progressive fibroid phthisis. Sputum abundant and increasing in amount.
	Dec., '14	.....	94	33	8	0	0	2.3	Slowly progressive fibroid phthisis. Sputum abundant and increasing in amount.
37	Jan., '15	9,000	110	22	3	0	0	4.4	Chronic, progressive. High temperature. About 150 c. c. sputum. Chills.
	Mar., '15	.....	90	55	7	0	0	1.4	Chronic, progressive. High temperature. About 150 c. c. sputum. Chills.
38	Nov., '14	.....	90	20	0	0	0	4.5	Chronic, progressive. High temperature. About 150 c. c. sputum. Chills.
	Dec., '14	9,100	125	22	2	0	0	5.6	Chronic, progressive. High temperature. About 150 c. c. sputum. Chills.
39	Jan., '15	.....	115	26	0	0	0	4.4	Chronic, progressive. High temperature. About 150 c. c. sputum. Chills.
	Mar., '15	.....	90	27	3	0	0	3.0	Chronic, progressive. High temperature. About 150 c. c. sputum. Chills.
40	Pulmonary tuberculosis	.....	130	30	3	0	0	3.9	Chronic, progressive. High temperature. About 150 c. c. sputum. Chills.
41	Pulmonary tuberculosis	9,400	127	16	1	0	0	7.4	Chronic, progressive. High temperature. About 150 c. c. sputum. Chills.
42	Jan. 5, '15	16,400	92	63	6	1	0	1.3	Chronic, progressive. High temperature. About 150 c. c. sputum. Chills.
	Jan. 8, '15	8,400	103	44	16	1	0	1.7	Chronic, progressive. High temperature. About 150 c. c. sputum. Chills.
43	Pulmonary tuberculosis	9,800	86	36	3	0	0	2.2	Chronic, progressive. High temperature. About 150 c. c. sputum. Chills.
44	Dec., '14	.....	120	40	5	0	0	2.6	Chronic, progressive. High temperature. About 150 c. c. sputum. Chills.
	Jan., '15	8,100	103	31	3	0	0	3.0	Chronic, progressive. High temperature. About 150 c. c. sputum. Chills.
45	" "	8,200	112	28	10	1	0	2.9	Chronic, progressive. High temperature. About 150 c. c. sputum. Chills.
46	" "	.....	90	27	10	0	0	2.4	Chronic, progressive. High temperature. About 150 c. c. sputum. Chills.
47	" "	.....	70	11	1	0	0	5.8	Chronic, progressive. High temperature. About 150 c. c. sputum. Chills.
48	" "	.....	103	55	7	0	0	1.6	Chronic, progressive. High temperature. About 150 c. c. sputum. Chills.
49	" "	16,000	95	21	9	0	0	3.1	Chronic, progressive. High temperature. About 150 c. c. sputum. Chills.
50	" "	6,300	70	31	8	0	0	1.8	Chronic, progressive. High temperature. About 150 c. c. sputum. Chills.

smear preparations stained with Wright's or Hasting's modification of the Romanowsky stain were used in every case.

In making the examinations of the stained specimens the writer became impressed with the difficulty which often arose in determining whether a given cell rightly belonged in Class II, III, IV, or V. It was, on the other hand, usually an easy matter to differentiate between cells of Class I (immature cells whose nuclei had not yet divided), and the more mature cells of the other classes which showed at least one true interlobular division. The study of the cases has, in the opinion of the writer, fully justified the plan of considering all undivided nuclei (Class I), as immature cells, and all divided nuclei, (Classes II, III, IV, and V), as mature cells, and of judging the degree of the "shift to the left" accordingly. The "Arneth index" as ordinarily used, has referred to the ratio between the number of cells which fall under Classes I and II, and those in Classes III, IV, and V. In the writer's opinion, however, a preponderance of the really immature leucocytes of Class I as opposed to all the other classes, is of more significance than is any variation between the proportion of the cells of the remaining classes, and it is believed that evidence in support of this opinion will be found in the data herewith presented.

The blood of forty-seven people was studied. Of these, nine were normal so far as infectious disease was concerned, seventeen were suffering from nontuberculous infections, and the remaining twenty-one, from pulmonary tuberculosis. The detailed results are given in the accompanying table.

In attempting to draw conclusions from the data presented, it may be well to review briefly some of the known facts regarding the activities of the polynuclear leucocytes. In health, the number of leucocytes in the circulating blood is maintained at a figure which varies between 5,000 and 10,000 per cubic millimeter. Of these, 60 to 70 per cent are polynuclears. Like most other cells, these leucocytes must be constantly dying or being destroyed, and as constantly replaced by new ones arising by cell division of their progenitors, the myelocytes of the bone marrow. In disease, the output of the bone marrow may be increased in two ways. First, it may be increased as the result of direct stimulation of the marrow by a toxic substance circulating in the blood, as in an acute streptococcus infection. In such a case the number of leucocytes in the circulating blood is markedly increased, and as the increase must be due to newly formed cells, a *slight* "shift to the left" of the Arneth formula occurs. Second, the output of the bone marrow may be augmented as a result of increased destruction of leucocytes. In such a condition as a tuberculous cavity in the lung the bone marrow is not specifically stimulated, but is merely attempting to replace the leucocytes which are constantly being destroyed or cast off. In this way the proportion of immature forms in the blood may be greatly increased, a *marked* "shift to the left," though no leucocytosis or even, indeed, a leucopenia results.

The toxin of the tubercle bacillus does not typically attract the polynuclear leucocyte. The histological work of Mallory clearly demonstrates that it is the endotheliocyte that is specifically attracted into the lesions of this disease. Furthermore, in tuberculosis a leucocytosis does not occur. When, however, the



tubercles become extensive, and necrosis and softening of the lesions occur, polynuclears invade the necrotic areas in very large numbers, apparently specifically attracted by the products of necrosis. When cavities are formed, and a large amount of sputum is discharged, enormous quantities of leucocytes are used up by actually being cast off from the body. The extreme "shift to the left" seen in such instances is illustrated by Cases 36 and 38 in the preceding tabulation. The bone marrow, stimulated by the abnormal withdrawal of leucocytes from the circulation, is forced to put out a large number of young cells into the blood stream.

When the increased demand on the bone marrow for polynuclear leucocytes has remained at the same point for a long time, hyperplasia occurs, as it does in the case of other overworked tissues. This is shown by an extension of the red bone marrow into the shafts of the long bones. As a result of this formation of new bone marrow the supply of leucocytes is increased to meet the demand, and it is no longer necessary for the marrow to put out immature cells. Under such conditions, with the extension of the bone marrow, the Arneth picture may return to normal, as is illustrated by Cases 28, 31, and 33, patients who, though casting off large numbers of leucocytes in the sputum, show a normal Arneth picture. The extension of red bone marrow to the long bones was observed in the six necropsies in which examination was made, all of them on fatal cases of pulmonary tuberculosis. In these instances the marrow of the femur was found on histological examination, to be producing leucocytes.

In nontuberculous infectious disease the "shift to the left" is, as in tuberculosis, evidence of an excessive demand on the bone marrow, or, in other words, it is evidence that an excessive number of leucocytes are being destroyed. For example, in Case 16, lobar pneumonia, the demand for leucocytes is, naturally, excessive, since large numbers are required to combat the infection in the lung, and they are continuously being cast off in the sputum. In this instance the ratio of immature to mature cells reached 4.2. Cases 10 and 13, in contrast, illustrate the fact that although moderate toxemia may exist as shown by the leucocyte count and temperature, if polynuclear leucocytes are not attracted into the lesions in large numbers, the Arneth picture may remain normal. Case 17 is of especial interest. On March 3, 1915, the patient evidently had a septic pneumonia resulting from a postoperative embolism. At this time she showed clinical evidence of marked toxemia. The leucocyte count of 31,000 is evidence of a strong bone marrow reaction to the toxemia, and there is also a well marked "shift to the left" of the Arneth picture. Five days later, although the clinical condition was somewhat improved as a result of the evacuation of large quantities of pus by coughing, and although the white count had fallen to 16,000, the demand for leucocytes had apparently increased, and the "shift to the left" was more marked. On March 12, with an apparent extension of the abscess, the beginning of gangrene, a "septic temperature," and extreme prostration, the bone marrow reaction seemed poor, leucocyte count only 12,000, and the Arneth "shift to the left" was extreme. After operation on March 14, improvement in the general condition occurred, and on March 26, although the maxi-

imum temperature was only  $100.6^{\circ}$ , the "shift" in the blood picture was still extreme. This was probably because fairly large quantities of pus were still draining from the operative wound. The record for April 22 showed a marked clinical improvement and a return to normal of both the temperature and the Arneth picture, although the leucocyte count still remained elevated. This case appears to be a good illustration of the fact that leucocytosis is evidence of toxemia: i.e., toxic stimulation of the bone marrow; while on the other hand, the appearance of immature polynuclears, the "shift to the left," denotes excessive destruction and, therefore, excessive mobilization of leucocytes, and has no relation to toxemia.

In judging the value of the Arneth picture, it is necessary to keep in mind this conception of its meaning. In early tuberculosis of the lungs a "shift to the left" must mean necrosis and extension of the lesion, and therefore activity of the process. Here the study of the picture should prove of value. In Cases 45 and 46 such a "shift" was the earliest recognized sign of impending progression of the disease. In advanced pulmonary tuberculosis the extreme "shift" seen in the progressive cases is accompanied by such definite clinical evidence of activity that here its value is not great. When the disease is very slowly extending, as in chronic phthisis, there seems to be a marked variation in the picture as shown in Cases 31, 32, 33, and 34, without evident change in the clinical condition. Here also it is of little use. In nontuberculous disease, as the leftward "shift" indicates excessive mobilization and destruction of leucocytes, the study of the picture may be of use in determining the nature of an obscure infection, and in indicating the presence of large collections of pus. It seems certain that the Arneth picture cannot be considered an index of the resistance of the individual to infection.

#### SUMMARY AND CONCLUSION.

1. The presence of a high percentage of immature polynuclear leucocytes in the circulating blood (Arneth's "shift to the left") is evidence of excessive loss of polynuclear leucocytes from the blood stream, and an attempt on the part of the bone marrow to make good the loss.

2. It does not indicate toxemia, nor is it an index of the patient's resistance to infection.

3. Leucocytosis, on the other hand, is evidence of toxemia and of direct stimulation of the bone marrow by certain toxins. A moderate leucocytosis may exist without causing an appreciable change in the Arneth picture.

4. In pulmonary tuberculosis the demand for leucocytes indicated by a "shift to the left" means necrosis, and extension of the lesion. In early stages of the disease this may be of considerable value in immediate prognosis, and even, at times, in diagnosis. In advanced disease which is slowly progressing, as the demand for leucocytes apparently varies from time to time, the count appears to be useless in estimating the prognosis. In rapidly progressing tuberculosis the unmistakable clinical evidence of activity renders the study of the blood picture superfluous.

5. In nontuberculous disease the evidence of excessive mobilization of leucocytes may be of value in doubtful cases to determine the presence of large collections of pus.

## A SIMPLE METHOD OF PLOTTING CHARTS\*

BY JOHN R. WILLIAMS, M.D., ROCHESTER, N. Y.

WITH the advent of precise methods of investigation in the medical sciences, the use of the graph as a means of conveying comparative mathematical information has become more and more common. The reason for this is very obvious. The graph, when properly arranged, shows at a glance what tables of figures will reveal only after long and patient study. Moreover, the relationship of one set of data to another and the general perspective of a study amenable to mathematical treatment can better be comprehended when plotted as a line drawing than when buried in a maze of tabulations.

One serious difficulty in the way of preparing graphs for hospital and laboratory use is that it requires the services of some one especially trained in this kind of work and such individuals are rarely to be found in the personnel of scientific institutions. Moreover, the labor of preparing graphic charts as ordinarily done, both as to time and expense, is prohibitive except in extraordinary instances.

In the writer's hospital service, where a number of diabetics and nephritics are under observation, it has been found desirable, both for purposes of study and teaching, to employ charts. For the above mentioned reasons, it has been impracticable to employ a draughtsman for routine work. The necessities of the situation, therefore, led to the development of the following method which answers the important requirements of most workers. A very elaborate chart can be made in an hour or two by an office assistant which, if drawn by a draughtsman in the usual manner, would take from one to two days. The materials are inexpensive; the printing and hatching are uniform; colors can be used in place of cross hatching when desired.

After much experimentation, a cross section paper was selected which may be purchased in sheets measuring sixteen by twenty-one inches. The bold ruling on this paper forms inch squares and the fine ruling one-tenth squares. A paper ruled to form half inch squares with the fine lines ten to the inch would be much better but this is not to be had, accordingly we have had the sheets reruled so as to have the bold lines one-half inch apart. A blank chart was then prepared containing all the words and figures which would appear on every chart. (See Fig. 1.) Blank spaces were left so that syllables, words, or numbers could be added as desired. On another sheet of paper, were drawn all the words and figures appropriately sized and spaced, which one might wish to add to a chart. These were so arranged that they could readily be cut out as needed. On another sheet were drawn eight different types of cross hatching, each one-half inch wide and of lines of sufficient boldness as to permit reduction for reproduction. (See Figs. 2 and 3.) From each of these, zinc etch-

\*In the development of this method of plotting, I wish to acknowledge valuable aid and advice of Mr. Frederick W. Fisher and Mr. F. A. Miller of the Engineering Department of the Rochester Railway and Light Company and Mr. C. W. Norwood, draughtsman.

ings and electros were made. From the large electro, the charts were printed. The other two electros containing the miscellaneous words and numbers and the cross hatching were printed on white gummed paper.

When it is desired to prepare a chart, figures are cut out of the gummed sheet to make the patient's case number and pasted in the proper place. If the patient is a female, the syllable "fe" is prefixed to male, likewise the words "mild," and "severe" may be placed before the word diabetes to indicate the

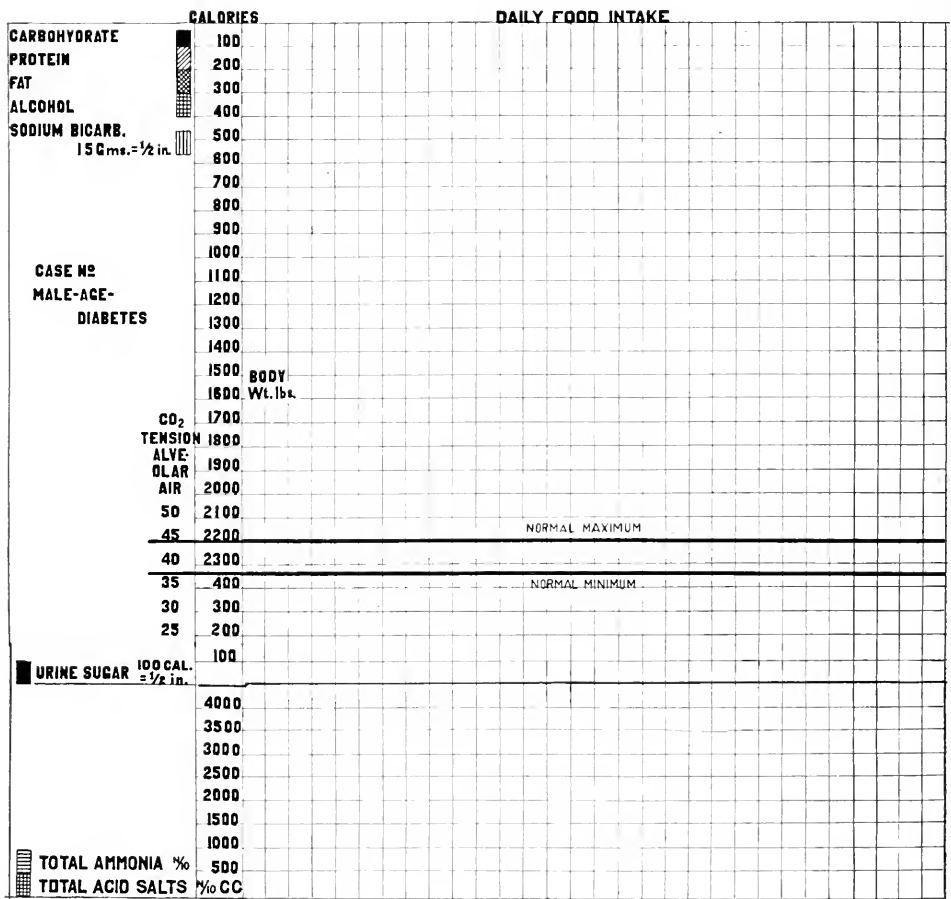


Fig. 1.—Blank chart used for plotting the metabolism of a case of diabetes mellitus.

severity of the case and the words "fatal" or "improved" to indicate its outcome. The date line is similarly added, the numbers being so spaced and sized as to fit each column which may represent a day of observation. The cross hatching is cut into strips and then into lengths to represent the quantities desired and these pasted in their appropriate columns. See finished chart, Fig. 4.

All of the trimming and cutting is done with a small inexpensive photographer's trimming board. To facilitate computations and reduce error to the minimum, a scale was prepared and attached to the trimming board which reads in grams but measures in calories. The scale has a double row of divi-

sions. The upper one is plotted for carbohydrates and proteins which have the same caloric value, one gram of either being equivalent to four and one-fourth calories. The lower line of divisions of the scale is arranged similarly for the fats, a gram of which is equivalent to nine calories. Accordingly, if one wished to plot the diet of a patient for several days, he would take from the hospital record the number of grams of carbohydrates, proteins and fats eaten each day. A piece of the cross hatching paper intended to represent carbohydrates would be placed on the cutting board at the figure representing the number of grams eaten and then cut. This piece, when moistened and stuck in its proper

**1916 1917**

**1 2 3 4 5 6 7 8 9 10 11 12 13 14**  
**15 16 17 18 19 20 21 22 23 24 25 26 27 28**  
**29 30 31 JAN. FEB. MAR. APR. MAY. JUN. JUL.**  
**AUG. SEP. OCT. NOV. DEC. FE MILD SEVERE FATAL**  
**IMPROVED 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0**

Fig. 2.—Words and numbers used in plotting a case of diabetes mellitus printed on gummed paper to be cut out and pasted on chart as needed.

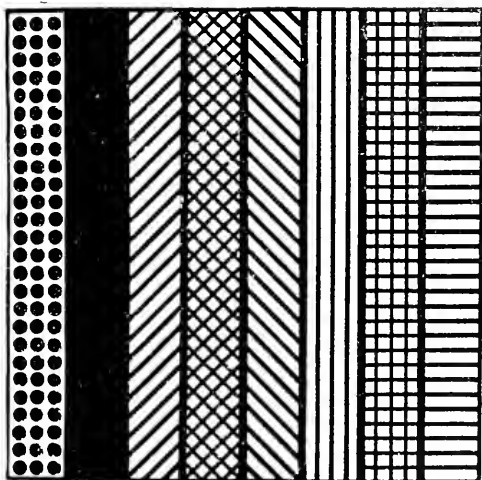


Fig. 3.—Eight types of cross hatching printed on gummed paper to be cut up and pasted on blank chart as needed. A simple way of avoiding the labor of draughting.

place on the chart, would then represent in length the number of calories in the number of grams of carbohydrate in question. The worker would be spared the labor of computation and the chance for error would be reduced to the minimum. Similarly, proteins and fats may be calculated and plotted. The total length of the cross hatching would thus represent the total number of calories. In the plotting of diabetic charts, the urine sugar is also plotted in calories so that the balance between intake and outgo may be seen at a glance. The weight line and curve of carbon dioxide tension of alveolar air are inked in with a ruling pen very quickly.

The accompanying figures illustrate the application of the idea to the plotting of the metabolism of diabetes; the method, however, lends itself readily to many other diseases, particularly nephritis, where it may be desirable to graphically compare the nonprotein elements of the blood with those in the urine and the relation of salt and food metabolism thereto.

The expense of preparation for making the charts was comparatively small, approximating fifteen dollars for one hundred charts, sixteen by twenty-one inches each. Thus the materials required to produce the chart shown in Fig.

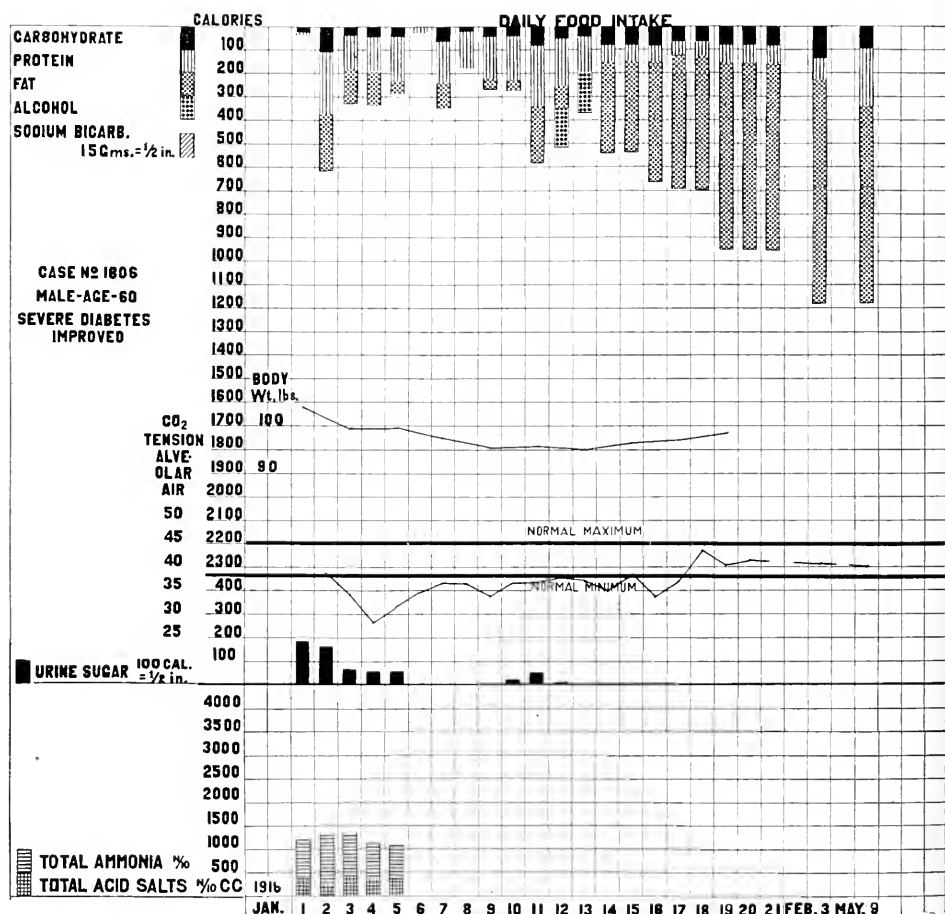


Fig. 4.—Showing practical application of the chart to a case of severe diabetes of long standing. This patient was excreting sugar and was in a state of marked acidosis although taking very little food. When the protein intake was reduced the patient immediately began to improve and in a short time was able to eat much more food than formerly with the same protein intake which before had caused injury.

6 cost approximately fifteen cents plus the labor of an assistant for about two hours.

The salient features of the method are:

1. The printing on standard sheets of cross section paper all the fixed words and characters which will be common to all charts. This usually can best be done by having them first drafted, and zinc etchings and plates or electros made therefrom.

2. The printing on gummed paper of all the common words and characters which the plotting of a given set of data will require. These to be of the proper size and spacing to fit in the chart so as to give uniformity.
3. The printing of several types of cross hatching on gummed stock so that it may be similarly used.
4. The use of a photographic trimming board to smoothly and correctly cut the words, characters, and hatching used and the attachment thereto of a scale to facilitate calculations and to minimize errors.
5. The facility and wide application of the method and its relatively slight cost.

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## MACROPHAGES IN FECES IN ACUTE DYSENTERY\*

BY KENNETH M. LYNCH, M.D., CHARLESTON, S. C.

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IN the early part of 1916 my attention was called to several cases of acute dysentery in the Roper Hospital, of Charleston. A fairly typical example of these is the following case.

J. M. R., white male, age 20 years, mechanic, was admitted on Jan. 10, 1916. His previous history was negative of any connection with the present illness. He had had no similar attack. About midnight of January 4th he had developed a severe diarrhea accompanied by pain throughout the abdomen. This condition had become worse and he was passing twelve to fifteen stools a day before admission, at first of a watery character but later becoming mucinous and bloody. He said he had a good appetite, but when he ate anything, it would pass through directly. His tongue was coated and furred and of yellowish brown appearance. His abdomen was generally tender on palpation. His temperature was slightly subnormal in the morning and averaged a degree and a half rise in the evening. There was no sign of any involvement outside the alimentary canal.

With no treatment besides rest in bed and the administration of bismuth subnitrate by mouth he improved rapidly, the abdominal tenderness decreased, the pain disappeared, the number of stools decreased to two a day, his temperature remained normal or slightly subnormal, and he was discharged on January 15th.

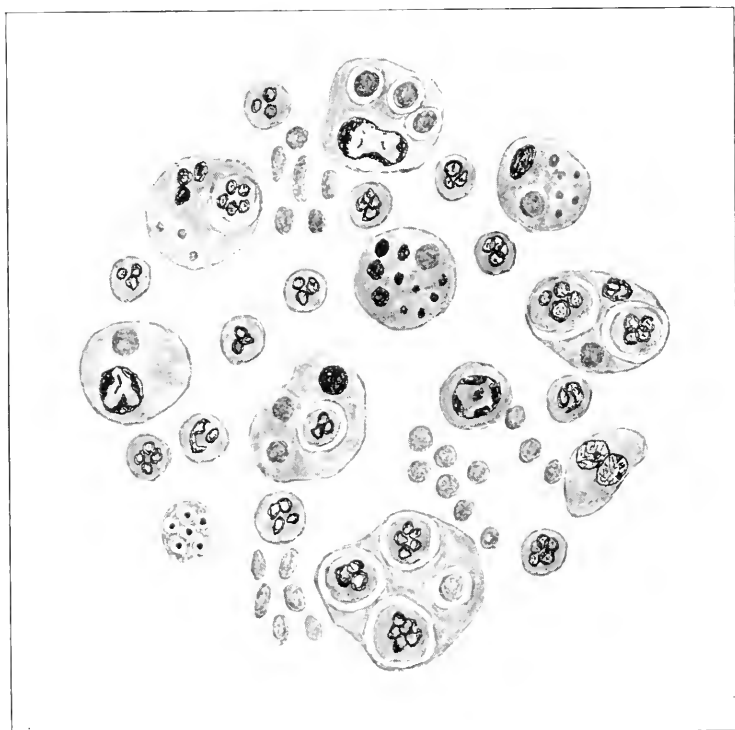
The first stool examined was of the appearance of thick blood-stained mucus. In a fresh warm wet preparation there were large numbers of polynuclear leucocytes and red blood cells, many active *Trichomonas* and an occasional *Entamoeba coli*. In addition there was a considerable number of round or ovoid cells, varying in size from twenty-five to forty-five microns, nonmotile, with densely granular cytoplasm, and a large ring-form nucleus. The nucleus usually had a definite refractive membrane and from one to four refractive granules clinging to its inner surface or free in the plasma. In some of the cells there were two of these nuclei, some had apparently no nucleus and in some

\*From the Department of Pathology and Research Medicine of the Medical College of the State of South Carolina.

it appeared to be represented by a group of large refractive granules without a limiting membrane.

Many of these large cells contained from one to three or four erythrocytes, and as commonly, from one to three polynuclear leucocytes, some containing both, usually within distinct vacuoles. Some of these engulfed erythrocytes were decreased in size and in some of the cells small bodies of the appearance of erythrocytes were seen. The engulfed leucocytes were commonly of the same appearance as the extracellular, but in many of the cells were refractive particles which were judged to be remnants of leucocytic nuclei.

Preparations stained by Heidenhain's iron-hematoxylin and by Leishman's methods confirm the observations made on the unstained specimens. In the



Macrophages in feces in acute dysentery. Case of J. M. R., Jan. 12, 1916.  
Heidenhain iron-hematoxylin stain.

stained specimens the broken up as well as the preserved red blood cells and leucocytes are of the same staining properties as those outside. Stained with hematoxylin the cytoplasm, and nucleus of the phagocytes are of the same color as in the leucocyte; but with Leishman's stain, the cytoplasm is blue and the nucleus purple.

While in the fresh preparation few bacteria were observed, in stained specimens they were fairly numerous, mainly engulfed by leucocytes. The predominant organism was a micrococcus.

The stool remained of bloody mucinous character for two days after admission; then as the number diminished, it gradually assumed the normal appearance.



the bacterial flora assumed the common proportions, and the cells gradually decreased in number until there were only a few leucocytes present. None of the large phagocytes was to be found after the second day, they disappearing before the red blood cells or leucocytes. The *Trichomonas* and *Entamoeba coli* remained in small numbers.

Based on the lack of active motility, the character of nucleus, the staining characteristics, and the early disappearance from the stool, this large phagocytic cell was judged to be a large wandering body cell similar in nature to Mallory's endothelial leucocytes. While these so-called endothelial phagocytes are known to play an important part in phagocytosis within certain organs and have been reported to exhibit activity in serous cavities and even within the blood stream, their entrance in numbers into the intestinal canal apparently through a more or less intact mucosa appears to be an occurrence of interest, from the standpoint of the study of this cell, and also in microscopic study of dysenteric feces.

Although a close study cannot fail to differentiate them, the occurrence of such phagocytes in a dysenteric stool of this character might easily lead to confusion with *Entamoeba* by the uninitiated.

While no definite determination as to the cause of the enteritis in these cases was made, I considered it as probably bacterial and possibly the micrococcus which was the predominant organism in the stool and extensively phagocytized by leucocytes.

## A FURTHER STUDY OF THE PATHOLOGICAL EFFECTS OF ATMOSPHERES RICH IN OXYGEN\*

BY HOWARD T. KARSNER, M.D., CLEVELAND, OHIO, AND J. EARLE ASH, M.D.,  
U. S. ARMY.

A PREVIOUS study† showed that in the case of the rabbit "atmospheres containing 80 to 90 per cent oxygen under normal barometric pressure produce in 24 hours, or more commonly 48 hours, congestion, edema, epithelial degeneration and desquamation, fibrin formation, and finally a pneumonia probably of irritative origin and to be described as fibrinous bronchopneumonia." The present study deals with a series of animals exposed to atmospheres containing 53 to 80 per cent oxygen. The purpose of the study is to determine whether or not these lower percentages operate to produce lung changes as rapidly or in the same degree as do the higher percentages previously reported. The apparatus, care of animals, use of contemporaneous controls, and pathological technique were the same as in the earlier work.

One rabbit was placed in the chamber with an atmosphere containing 53 per cent oxygen for 3 days, 16½ hours. A control was placed in the same sized cage with room air. Both animals were removed alive and apparently well. The autopsies showed no pathological alteration of lungs or other viscera.

Two rabbits were placed in the chamber with about 67 per cent oxygen for 3 days, 18½ hours. The usual control was employed. The animals were removed alive and apparently well. The lungs of the animals exposed to "high" oxygen showed moderate congestion associated with slight cloudy swelling of alveolar epithelial cells; there was neither desquamation nor exudation.

Three rabbits were placed in the chamber and stayed 11 days during most of which time the oxygen percentage varied between 60 and 70. For a few hours on the first day and again on the fifth day the oxygen content sank to normal; on the second day the oxygen rose for a very short period to 78 per cent. The animals were removed at the end of 11 days alive and apparently well. In the case of one of these animals, the lungs were perfectly normal except for a slight congestion, not more marked than in the normal control. Another animal showed marked congestion of the lungs with a small amount of edema within the alveoli as well as slight congestion of the abdominal viscera, not more marked than in the control. The third animal showed moderate swelling of the alveolar epithelium and a few alveoli showed desquamation of the swollen cells; there was, however, no fibrin formation, edema, or exudation. The abdominal viscera of all three animals showed slight congestion but not in greater degree than in the control.

Two rabbits were placed in the chamber with oxygen content of about 75 per cent. On the third and fourth days, however, the oxygen rose for a few hours to 90 per cent. They were then kept in room air for 4 days. Following

\*From the Nutrition Laboratory of the Carnegie Institution of Washington, Boston, and the Laboratories of Pathology of the School of Medicine of Western Reserve University, Cleveland, and the Harvard Medical School, Boston.

†Karsner, H. T.: The Pathological Effects of Atmospheres Rich in Oxygen, *Jour. Exper. Med.*, 1916, xxii, 149.

this they were placed in the chamber for 6 days with 74 per cent to 78 per cent oxygen, at the end of which time they were found dead. Both animals showed well marked pneumonia, cloudy swelling of the myocardium and marked congestion of the abdominal viscera.

Two rabbits were placed in the chamber with oxygen averaging about 75 per cent for 10 days. On the seventh day the oxygen sank to 44 per cent for a short time and later rose for a short time to 91 per cent. The animals were removed on the eleventh day, alive but weak and dyspneic. The lungs of these animals showed slight congestion but not in greater degree than in the control. The abdominal viscera showed well marked congestion.

It is apparent that a short exposure (3 days, 16½ hours) to 53 per cent oxygen produced no apparent change in the rabbit's lung. Exposure to 67 per cent oxygen for a short period (3 days, 18½ hours) produced extremely slight changes, so slight as to be distinguished only after most careful examination. Three rabbits exposed for 11 days to atmospheres between 60 per cent and 70 per cent oxygen showed very slight evidences of pulmonary irritation but no true inflammatory reaction. Exposure for 10 days to 75 per cent oxygen showed an early pneumonia. The same was true of 2 additional animals exposed to approximately the same atmosphere for 6 days but complicated by accidental exposure to air of a higher percentage (90 per cent) 4 days before the last experimental period.

It will be seen that there is considerable difficulty in maintaining a constant oxygen percentage in the chamber during the long periods demanded in this series. On the other hand, departures from the desired constant were of brief duration and with the exception of one pair of animals apparently of no importance in the outcome of the experiment.

This study confirms the earlier study in regard to the inter-relationship of pneumonia, myocardial cloudy swelling, and the congestion of the abdominal viscera. It is shown that animals exposed to atmospheres containing less than 80 per cent oxygen and more than 60 per cent oxygen withstand the exposure for a considerably longer period than those animals exposed to percentages greater than 80 per cent. This must not be interpreted as indicating that 80 per cent is a critical point in the scale of percentages. Reference to the previous paper will show that this point was selected merely as a matter of convenience.

The correlation of this study with its predecessor will indicate that in a very broad general way, and modified probably in many ways by uncontrollable factors in the individual animals, the higher the percentage of oxygen in the atmosphere, the earlier do pneumonia and the secondary changes in the other organs make their appearance.

## BLOOD PRESSURE IN THE AGED

### A Study of 150 Cases from 65 to 95 Years of Age.

BY L. M. BOWES, M.D., CHICAGO.

A GREAT deal of work is being done, in the study of blood pressure, at the present time. It is of special interest in the aged because so many of the diseases have characteristic findings, and it aids us, not only in differential diagnoses, but also in prognoses and treatment. It is because of this aid that I have made a study of these cases.

On account of the different methods and positions of taking blood pressure, I am giving a brief description of the technic which was employed in this work.

The patient was seated in a chair with the bared left arm partially flexed, supinated and resting on a table. The armlet was then applied to the arm on a level with the heart and adjusted to be read conveniently and quickly. The bell of the stethoscope was applied just below the flexure of the elbow and a little to the inner side. The armlet was then gradually inflated until a point 10 mm. Hg. above that at which all sound disappeared had been reached. The air was then allowed to escape, slowly, noting the point when the first sound appeared. The air was allowed to escape further until the pressure dropped from the point of maximum intensity to a dull tone. This point was recorded as the diastolic pressure. The difference between these readings was recorded as the pulse pressure.

On account of the great hardness of the blood vessels of some patients, it was very difficult to get a true and accurate reading, making it necessary to make repeated observations to correct or verify former findings. The artery below the flexure of the elbow of one patient was misplaced so that the reading could not be made by auscultation. This case is not included in this series.

In some patients who were apprehensive it was necessary to adjust the armlet for a little while before taking the blood pressure, and wait until they became accustomed to the procedure and relaxed sufficiently to make an accurate reading. When the patient became nervous, the systolic pressure was almost invariably raised 10 to 25 mm. Hg. When the observations were made too frequently the systolic pressure was often increased 5 to 10 mm. Hg. probably due to spasm of the vessel wall. As a large number of the aged tire easily, the readings were made as quickly as possible. It was noted that when they became tired during the observations, the systolic pressure fell 5 to 10 mm. Hg.

In one case the pressure in the armlet was maintained a little too long causing one of the small vessels in the anterior portion of the wrist to rupture, resulting in a slight hemorrhage extending over an area about four inches in length. The discoloration cleared up in about a week with no bad effects. The air must be entirely released between each separate reading and not maintained too long.

Seventy-five per cent showed an inequality of the blood pressure on the two sides of the body. Where this condition was constant, the arteries were always quite sclerosed. Of seventy-five per cent of those showing a difference of blood pressure in the two arms, an inequality was shown in the systolic pressure, and

sixty per cent in the diastolic pressure. There was an inequality of the diastolic pressures in only one instance where the systolic pressures were equal. Where there was an inequality of the systolic and diastolic pressures, seventy-five per cent were higher in the left arm.

The greatest difference shown was a man, aged 74, who complained of dizziness. He had a combined aortic and mitral regurgitation. The arteries were greatly sclerosed. The pulse was 50. On the left side the systolic pressure was 178, the diastolic pressure 104, and the pulse pressure 74 mm. Hg. On the right side the systolic pressure was 230, the diastolic pressure 104, and the pulse pressure 126 mm. Hg.

Engel believes that this sign may be useful in differentiating between primary nephritis and arteriosclerosis. The only instance in which I found this inequality in nephritis, was a man, aged 70, who complained of swelling of the limbs and pain in the back. The heart was normal and the arteries slightly sclerosed. The systolic pressure on the left side was 232, the diastolic pressure 115 and the pulse pressure 117 mm. Hg. On the right side the systolic pressure 190, the diastolic pressure 112 and the pulse pressure 78 mm. Hg. The urine was acid and the specific gravity 1030. Both albumin and sugar were present.

A man, aged 75, whose right leg was amputated at the junction of the upper and middle thirds, because of septic arthritis, had a systolic pressure of 142, a diastolic pressure of 85, and a pulse pressure of 57, on the left side, and a systolic pressure of 120, a diastolic pressure of 65 and a pulse pressure of 55 mm. Hg. on the right side.

In a case of senile dementia the blood pressure was equal. The patient was a woman, aged 67, with a normal heart. The arteries were greatly sclerosed. The systolic pressure was 144, the diastolic pressure 72, and the pulse pressure was 72 mm. Hg.

There were 100 women and 50 men examined in this series. The systolic and pulse pressures increased to the age of 85 and then decreased. The diastolic pressure did not vary much after the age of 70 (see Table I).

The blood pressure was higher in women except after the age of 90 (see Tables II and III).

TABLE I.—THE AVERAGE BLOOD PRESSURE OF BOTH MEN AND WOMEN.

Age.	Number examined.	Systolic pressure.	Diastolic pressure.	Pulse pressure.
65-69	32	151	82	65
70-74	39	160	86	73
75-79	38	166	86	79
80-84	27	175	84	83
85-89	7	170	90	77
90-94	7	142	81	61

TABLE II.—THE AVERAGE BLOOD PRESSURE OF THE WOMEN.

Age.	Number examined.	Systolic pressure.	Diastolic pressure.	Pulse pressure.
65-69	21	154	83	71
70-74	29	158	83	72
75-79	24	170	88	81
80-84	16	183	85	91
85-89	7	170	90	77
90-94	3	137	80	53

TABLE III.—THE AVERAGE BLOOD PRESSURE OF THE MEN.

Age.	Number examined.	Systolic pressure.	Diastolic pressure.	Pulse pressure.
65-69	11	145	81	63
70-74	10	166	91	75
75-79	14	159	89	77
80-84	11	163	84	80
85-89	0	—	—	—
90-94	4	145	81	65

There were a few who had very high blood pressure without symptoms. They were going about their every-day duties in apparently perfect health.

There were some who simply complained of a morning headache, slight vertigo, numbness or tingling of the hands and feet.

A third group presented the more severe symptoms of nephritis, aortic regurgitation, cerebral hemorrhage, and arteriosclerosis.

There were 22 who had systolic pressures of 200 mm. Hg. or over. Thirteen had arteriosclerosis alone, 3 had cardiac lesions, 2 had nephritis, 2 had cerebral hemorrhage and cardiac lesions, 1 cerebral hemorrhage, and 1 nephritis and a cardiac lesion. The highest systolic pressure was 270 mm. Hg. This was a case of nephritis.

There were 28 who gave diastolic pressures of 100 or over. Sixteen had arteriosclerosis alone. Seven had cardiac lesions, 2 had cerebral hemorrhage and a cardiac lesion, 2 cardiac lesions and nephritis, and 1 had cerebral hemorrhage.

There were 30 who had pulse pressures of 100 or over. The highest pulse pressure was 150, observed in a case of aortic regurgitation. Sixteen had cardiac lesions, 7 arteriosclerosis alone, 3 cerebral hemorrhage, 3 nephritis, and 1 had nephritis and a cardiac lesion.

All cases of nephritis had very high blood pressures. Where there was a continuous hypertension, nephritis was present, or the findings of chronic interstitial nephritis presented themselves in the majority of cases. Those escaping were the subjects of cerebral hemorrhage.

The blood pressure was of no practical value in any of the heart lesions except aortic regurgitation. In these cases the pulse pressure was always very high due to the low diastolic pressure or both high systolic and low diastolic pressures. The following case is an illustration. The patient was a woman, aged 77, who had a compensated aortic regurgitation. The systolic pressure was 214, the diastolic pressure was 75, and the pulse pressure was 139 mm. Hg.

Where there was a combined nephritis and organic heart disease, the blood pressure was very high. In these cases of hypertension the heart had hypertrophied sufficiently to maintain the tension.

Where there was broken compensation accompanied by rapid and irregular heart action, the tincture of digitalis was used with good results. I have never seen injurious results follow its careful use in cases of broken compensation.

The systolic, diastolic, and pulse pressures were high in all cases of cerebral hemorrhages. In all cases of very high blood pressure, especially diastolic, after heart and kidney lesions were eliminated, the proper precautions were carried out to safe-guard against hemorrhage. The pressure dropped slightly following hemorrhage, but not permanently.

In one woman, aged 80, who is not included in this series of cases, the hemorrhage was from the eyeball. A year later there was a rupture of the other eyeball with hemorrhage. Infection and then meningitis followed.

Some of those with the hardest arteries presented normal blood pressure and appeared in good health. Some with normal radial arteries had hypertension. Only about 25 to 30 per cent with marked arteriosclerosis presented high blood pressure. A high pulse pressure was common.

Acute enteritis caused a fall of 5 to 15 mm. Hg. The blood pressure was low in cases of myocarditis, failing heart, and cerebral embolism. The latter fact may be of value in differentiating between cerebral hemorrhage and cerebral embolism.

The blood pressure may be low and still present no indication. A man, aged 65, who was attending his business every day had a systolic pressure of 100, a diastolic pressure of 60 and a pulse pressure of 40 mm. Hg.

A low pulse pressure was associated with myocarditis or a failing heart; and a sustained pulse pressure of 100 or over usually resulted in myocarditis or a failing heart.

In most cases of bronchitis there was a failing heart with lowering blood pressure. One woman, aged 81, had hypertension.

There were four cases of carcinoma, three of which had normal blood pressure. Two had involvement of the right breast, and one, the nose. The fourth case was a woman, aged 79, who had a carcinoma of the stomach with the characteristic coffee ground vomitus. The systolic pressure was 232, the diastolic pressure 130, and the pulse pressure was 102 mm. Hg.

Obesity did not seem to influence the blood pressure, as one woman, aged 76, who weighed about 270 pounds, had a systolic pressure of 160, a diastolic pressure of 80, and a pulse of 80 mm. Hg.

#### CONCLUSIONS.

1. Only repeated readings of both systolic and diastolic pressures are of value, and both arms should be used for observations in old people.
2. Inequality of the pressures of the two sides is frequent in arteriosclerosis.
3. There may be a high or low blood pressure in arteriosclerosis; the pressure falling with involvement of the heart muscle in the process of fibrosis resulting in chronic myocarditis.
4. High systolic pressure associated with high diastolic pressure indicates cerebral hemorrhage or nephritis.
5. A sustained hypertension, both of systolic and diastolic pressures, indicates cerebral hemorrhage, while hypotension indicates cerebral embolism.
6. A sustained high systolic with a low diastolic pressure usually indicates cardiac trouble. A low diastolic pressure is common with aortic regurgitation.
7. A high pulse pressure is frequent in arteriosclerosis and aortic regurgitation; and a sustained high pulse pressure usually results in a failing heart.
8. A systolic pressure of 100 may not keep a man from his daily business.
9. A lowering blood pressure indicates a failing heart.
10. Acute enteritis lowers the blood pressure.

## LABORATORY METHODS

### THE DEMONSTRATION OF TREPONEMA PALLIDUM IN THE CEREBRAL CORTEX OF A CONGENITAL SYPHILITIC CHILD\*

BY CHARLES E. KIELY, M.D., CINCINNATI, OHIO.

THE following report has to do with the demonstration of the *T. pallidum*, by means of the Noguchi technic, in the central nervous system of a syphilitic child.

The clinical history is as follows, and for it I am indebted to the Pediatric Service of the Cincinnati General Hospital. The child (Hospital No. A-5469) was admitted to the hospital on August 13, 1916, suffering from a discharging sinus in the back. The family history was not obtained.

*Past History.*—The patient was born three days before admission and at the time of birth showed a tumor over the lower part of the spine, extending about two inches along the spinal column and containing a clear liquid covered by only a very thin membrane. This tumor was about six inches long and four wide. The tumor already showed signs of sloughing. Otherwise the child seemed normal.

August 20, 1916.—The patient had a purulent discharge from the back and the tumor had disappeared.

August 30, 1916.—Patient showed evidences of meningitis with bulging fontanelles and rigidity of the muscles of the back; and a subnormal temperature.

September 10, 1916, Patient died.

*Clinical Diagnosis.*—Spina bifida; meningitis.

*Autopsy Protocol (M.)*—The body was that of a white female child about 18 inches in length. Postmortem rigidity had disappeared and lividity was present. There was an over-riding of the bones of the skull. There were no teeth present in the mouth. Upon each heel there was an area measuring 3 cm. in diameter, which appeared to be an ulceration due to pressure.

Over the lumbar region there was a reddish ulceration that measured fully 5 cm. in diameter. Upon section through this, it was found that the dura mater of the cord presented itself immediately and the vessels of the dura and of the cord beneath were found to be inflamed. The dura was adherent to the under surface of the ulceration and to the spinal canal just adjacent, but did not protrude as an open wound.

No abnormality was found in the lungs, heart, spleen or kidneys. The liver was larger than usual, very dark red in color, soft, and on section the organ cut readily, the cut edge turning out and from it could be expressed a blood-tinged fluid.

On opening the skull the dura was not found to be unusually adherent. The brain was large and soft and on pressure there escaped from both lateral

\*From the Pathologic Institute of the Cincinnati General Hospital.



ventricles fully 500 c.c. of a clear, straw-colored fluid. The inner lining of the lateral ventricles was smooth and atrophic in appearances.

*Anatomic Diagnosis.*—Spina bifida; meningitis of the cord; hydrocephalus; hereditary syphilis.

When received by the writer the brain was in 10% formalin. In addition to the notes made at the postmortem table it may be said that the ventricles

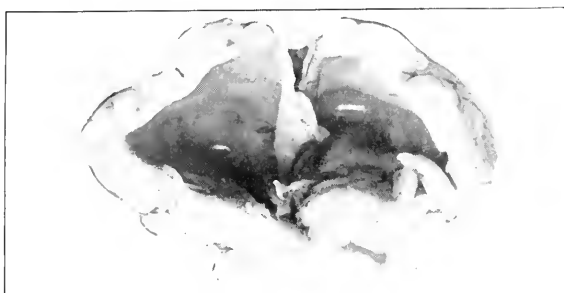


Fig. 1.—Gross photograph of the brain after hemisection in the coronal plane. The shell is decidedly thicker here than elsewhere as the section passes through the basal nuclei. The ventricles have been mechanically distended for photography.

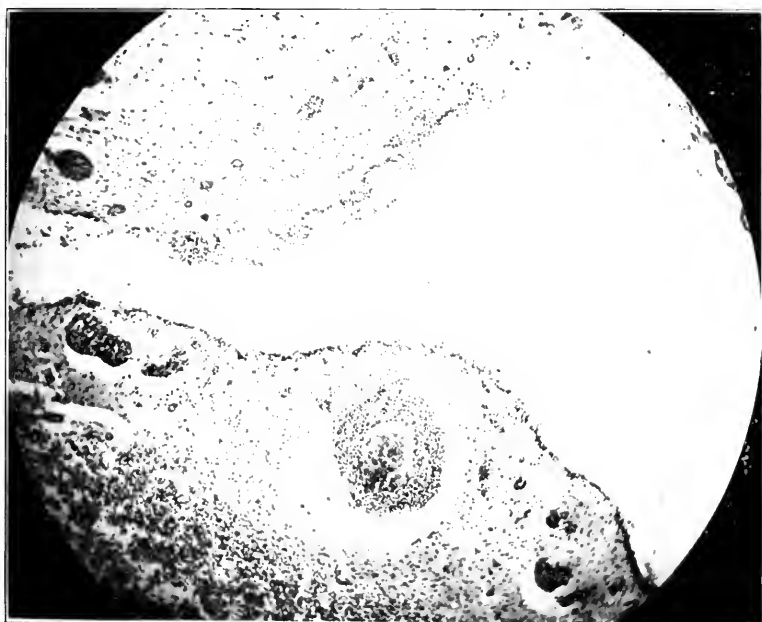


Fig. 2.—Section through the upper level of the pons. Microphotograph. The clear, roughly triangular area is the fourth ventricle.

were necessarily tremendously dilated and the substance reduced to a shell averaging about three-quarters of an inch (Fig. 1). The iter and the fourth ventricle were patent and not in the least distended. The choroid plexuses were reduced to mere fibrotic strands. The veins of Galen could not be recognized. Microscopically the plexuses are infiltrated with small round cells and

largely sclerotic (Fig. 3). The brain substance had a gelatinous feel and was extremely friable. Sections through the upper level of the pons showed a moderate thickening of the pia with small round cell infiltration. The reaction in the vessels was more marked; they were greatly engorged even to the fine capillary branches. The larger vessels showed a tremendous infiltration



Fig. 3.—Microphotograph of the choroid plexus.

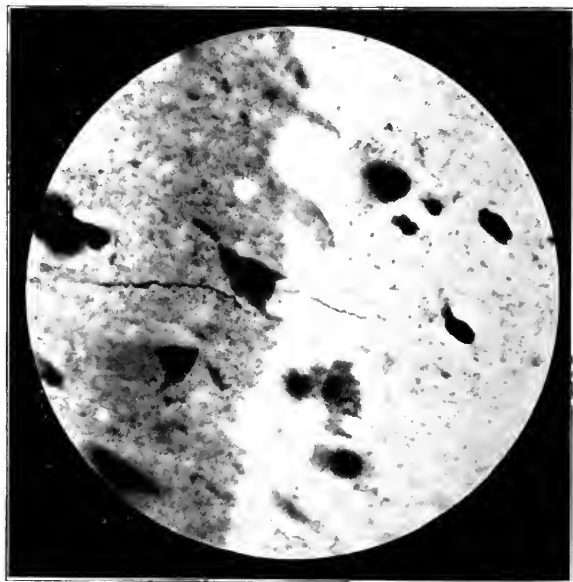


Fig. 4.—Noguchi preparation. Left of the nerve cell in the center of the field is a neuro-fibril. To the right is a treponema. Between this and the nerve cell is another treponema not in focus of this photograph but which to the eye was quite typical when in focus.

with small round cells (Fig. 2). The ependyma of the fourth ventricle was exfoliated to a considerable extent.

Sections through the entire thickness of the cortical shell were stained by the Noguchi technic. Treponemata were found with comparative ease and in rather large numbers considering their usual scarcity in nervous tissue. (Figs. 4 and 5.) These sections have been reviewed by Professors Wherry, Woolley, and Morris and the diagnosis sustained.

The mechanism of the hydrocephalus offers some difficulty. The lack of deformity in the iter and fourth ventricle preclude obstruction at the foramina of Luschka or Magendie. The iter was plainly patent. Experimentally hydrocephalus has often been produced by obstruction of the veins of Galen.<sup>1</sup> Such may have been the mechanism here. Transudation from the diseased vessels or tissues is a possible explanation and the one ascribed for such cases

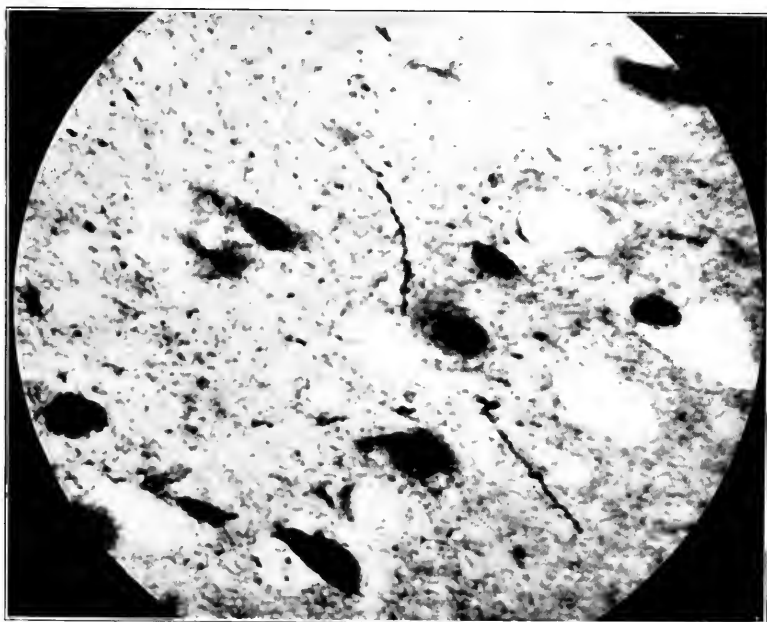


Fig. 5.—Noguchi preparation: a second field showing two treponemata.

by Nonne,<sup>2</sup> who says, "In many cases, a syphilitic arteritis is probably the cause of the hydrocephalus, and primarily also the cause of the congenital malformations produced by the hydrocephalus. The vessel disease promotes the exudation of lymphatic fluid in early intrauterine life, which causes a watery accumulation in the brain vesicles and the central canal."

The cord was unfortunately not secured in this case. There were no signs of acute meningitis in the portions of the central nervous system, i.e., the brain and brain stem which came into my hands. In the silver preparations there was no evidence of ependymitis.

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## A MACHINE FOR SHAKING BLOOD-MIXING PIPETTES\*

BY GEO. G. LITTLE, M.E., ROCHESTER, MINN.

TO eliminate all shaking by hand of the glass tubes used to determine the number of red and white corpuscles to a cubic millimeter in testing blood, a motor-driven machine was designed and built to duplicate the hand method as nearly as possible.

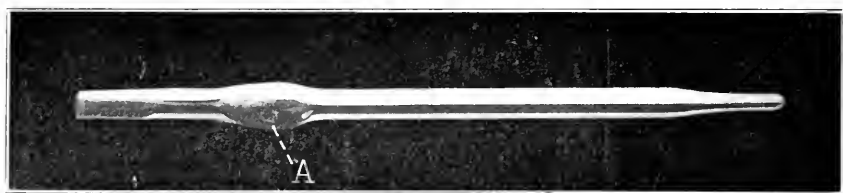


Fig. 1.—A, cube.

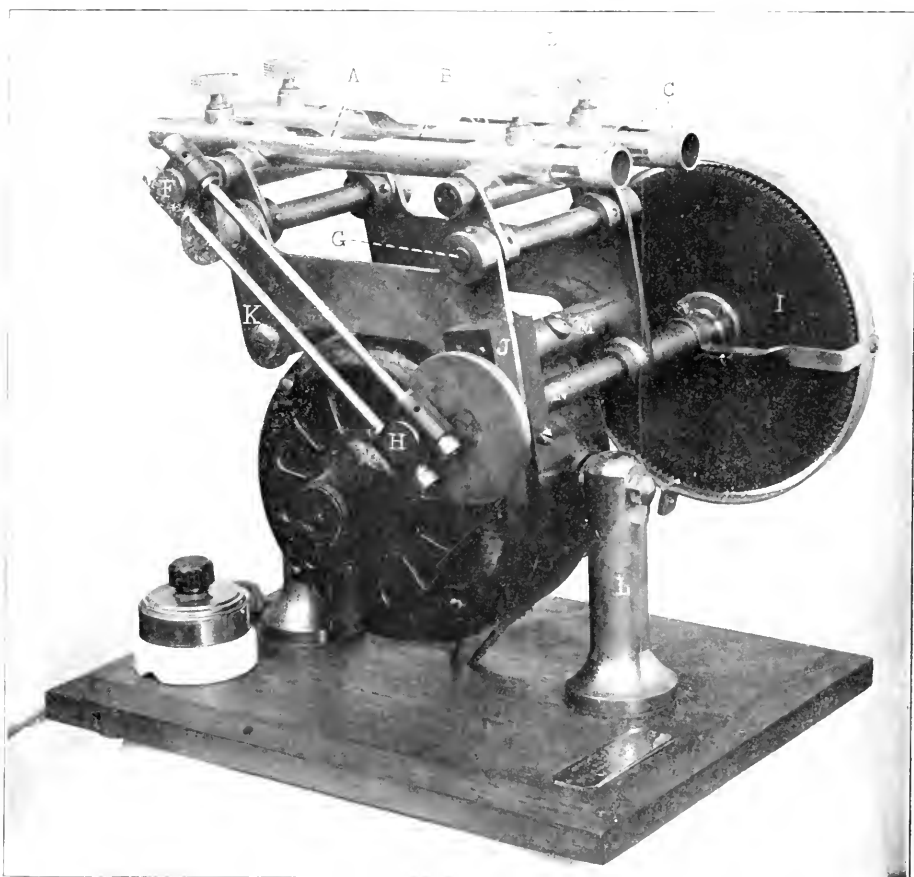


Fig. 2.—Machine for shaking blood-mixing pipettes. Baseboard 9x12 inches; height of instrument 9 inches.

\*From the Instrument Department of the Mayo Clinic, Rochester, Minnesota.

A study of the hand motion while shaking the tube was made to determine the exact movement, the number of strokes per minute, the length of time per tube and the possible saving of time from the use of a machine.

The hand movement showed the tube to be moving through an arc the radius of which was equal to the distance from the tube to the wrist, and the length of the stroke to be about two inches.

Two tubes are usually shaken at one time by the hand method. They are held endwise between the thumb and the first and middle fingers and given a rapid reciprocating movement lengthwise.

A pipette or tube (Fig. 1) has a bulb or enlarged portion near the middle containing a small glass cube as shown at (*A*). This bulb is open to the air through a fine hole about one-tenth of a millimeter in diameter. Through it a solution is drawn and into the solution a few drops of blood. The tube is then shaken to throw the cube from side to side through the mixture. In this manner the blood corpuscles are separated so that when distributed in a measured thin layer on a glass slide they can be readily counted by the aid of a microscope.

Fig. 2 shows the machine mounted on a small fan-motor. An idea of size is given by the motor and the large gear both of which are about six inches in diameter.

The pipette (*A*) is shown in position ready to be shaken.

The holder (*B*) is made of brass tubing. Lugs are soldered in place to hold thumb screws (*D*) which serve to secure the adjustable slides (*C*) in place while the tubes are being shaken.

The inner ends of the slides (*C*) contain rubber tips which receive the ends of the pipettes, pressing against and closing the holes and thus keeping the solution from being shaken out of the tube.

The holders (*B*) are secured to cross trunnions bearing in the upper ends of the short arms on the shafts (*G* and *E*) and are moved through an arc. The radius of the arc is one inch and the stroke one and one-half inches.

The wrist (*F*) carries the upper end of the connecting rod which transmits the motion of the wrist (*H*) on the crank shaft (*N*) driven by the gear (*I*) which meshes directly with the pinion on the motor shaft.

The bearing blocks (*J*) are made of fiber and are secured to the side frame (*K*). They support the crank end of the shaft (*N*) and are split at the lower ends and fitted with screws to take up the wear of the shaft.

Two space rods (*M*) secure the side plates in position on the motor as well as hold them in alignment.

The motor is supported by two standards (*L*) which are secured to the base board and have lead washers underneath to deaden the slight noise of the motor and gears.

The board is nine by twelve inches and a snap switch for starting and stopping the machine makes it complete.

The installation of this machine saves the services of one assistant.

# FURTHER NOTES ON A MODIFICATION OF THE NOGUCHI TEST\*

BY NORMAN E. WILLIAMSON, M.D., STOCKTON, CALIF.

THE results of 142 tests by the short method advocated in a recent article follow:

CLINICAL DIAG.	AUTHOR'S MODIFICATION OF NOGUCHI.	NOGUCHI OR WASSERMANN.	NUMBER.
Negative	Negative	Negative	86
"	Negative		35
"	Negative	Weakly positive (+)	1
"	Suspicious ±	Noguchi	
Syphilitic	+ +	Negative	5
"	+ +	+ +	5
"	+ +		2
"	+ +	Negative	2
"	+ +	Suspicious ±	1
"	Suspicious ±	Negative	2
"	Suspicious ±	Suspicious ±	1
"	Negative	Negative	2

For Noguchi and Wassermann tests the serum was inactivated. Acetone insoluble fraction was used as antigen. Sach's antigen would have made a better showing, but positives must be checked by a less sensitive antigen.

Noguchi antigen can be used with cholesterin and makes an exceedingly sensitive antigen which can be used with active blood in my modification. It is not more hemolytic or anticomplementary than the antigen from which it is derived. I use 0.5 c.c. of 1% cholesterin in absolute ethyl alcohol added to 1 c.c. of the methyl alcohol solution of the acetone insoluble fraction. One part of this to 9 parts of salt solution makes the emulsion. I used 0.1 c.c. of this in 11 tests and had one positive (+ +) reaction that was negative by Noguchi antigen. This case gave a syphilitic history. I consider that negatives with this antigen have a decided value; positives require investigation.

I now use test tube racks with 4 rows of holes. I obtain 0.12 c.c. of blood from the ear and add it to 3.88 c.c. of the salt solution, containing 4 parts per 10,000 of sodium citrate, in a wide test tube. This facilitates shaking. One c.c. is added to each of the four tubes. In the front row Noguchi antigen with cholesterin is used; in the next Noguchi antigen; the third contains the anticomplementary control; the 4th contains nothing during the first incubation to determine fragility of cells; later 3 units of amboceptor are added, as in the other tubes, as a measure of human complement.

When such fresh blood was used I was unable to detect complementary action in 18 cases, in the presence of 1½ units of amboceptor. When 3 units are used there is a faint tinge of hemolysis in about 20% of the cases. If the tube with antigen shows no more color than the fourth tube, complete inhibition of the guinea pig complement can be recorded. So far I have not seen a case in which the human complement was a factor of any force.

\*From the State Hospital, Stockton, California.

Tchernogubou used sodium citrate and utilized the human complement to hemolyze the patient's cells, as in this test. This cannot be an accurate gauge of the amount of complement fixed, and requires an enormous amount of amboceptor. The action of the sodium citrate on amboceptor seems to have been overlooked. This I will refer to again.

Von Dungern defibrinated blood, which of itself would produce some hemolysis. He used the patient's cells and disregarded human complement. He used an alcoholic extract as antigen which would give nonspecific proteotropic reactions. He used 0.1 c.c. of blood which is a large amount for active blood, and which would moreover occasionally contain an appreciable amount of complement.

I was convinced by my original experiments that sodium citrate did not affect the substance in syphilitic blood that was responsible in the presence of antigen for the complement fixation or absorption, and the results here tabulated show that this observation is correct. It was necessary to determine the action of the citrate on the other factors in the test. That cells are not affected has been shown by daily experience in obtaining them for complement fixation. Guinea pig serum 14 hours old was titrated as follows: to one portion was added 4 parts of salt solution; to another portion was added 2 parts of salt solution and two parts of 1% citrate salt solution. This makes about the proportion of citrate which would have been added to the whole blood to prevent clotting. There is this difference, however, that the calcium has been used in the clotting of the blood and the citrate is an excess. The two portions of 20% complement were titrated in parallel rows in the usual way with 1 unit of amboceptor and 1 c.c. of 1% suspension of washed human cells; 0.01 c.c. was the interval between tubes. The salt solution complement showed a unit of 0.08 c.c. The citrated complement reached maximum hemolysis at the same point, the hemolysis being almost, but not quite, complete. There were just a few more cells in the tubes containing more complement. This showed that there was no effect on the complement, but did suggest a deleterious action on amboceptor.

Fresh guinea pig blood was added to an equal quantity of 1% sodium citrate, centrifugated, and decanted. It was at once tested for complement. It was present but not strong. The next day the complement was strong. Just enough 1% calcium chloride was then added to a portion to produce clotting. On separation of the clot the complement was found to be unaffected. Calcium chloride in excess of this amount was anticomplementary, as is known. It may be possible that excess of calcium is one of the factors concerned in the anticomplementary action of some sera. None of the bloods used in the test in question have appeared to have a marked anticomplementary action. This may, however, be due to the freshness of the material.

Adding measured amounts of 1% sodium citrate in making the dilution of amboceptor it was found that one unit of amboceptor was destroyed by 0.2 c.c. of 1% citrate salt solution. That this was not caused by change in osmotic tension was shown by adding salt solution in the same concentration. It took 0.5 c.c. to have the slightest detrimental effect on the reaction. The citrate used in the test has united with calcium and very little is free. The extra unit of amboceptor

found to be of advantage in this test is to overcome the antihemolysin present in many bloods for the patient's own cells; to produce a quick reaction for early reading; to anticipate by such early reading the strengthening of the human complement. I believe, moreover, it assists in eliminating false positions. Patients showing suspicious reactions should be made the subject of thorough investigation. Five out of seven were shown by careful study, including examination of spinal fluid, to be in all probability free from syphilis.

After the tubes have stood for many hours a clot forms in each tube. This suggests the possibility of a chemical union of citrate and amboceptor, by which the calcium is set free. If it were due to calcium in excess in the guinea pig serum added, the action should be more prompt.

All patients admitted to this hospital by 10:00 A. M. on Mondays and Thursdays are examined by this method and a report made to the Clinic at 2:00 P. M. of these days. Strong positives and decided negatives have a great value in the study of the case. The possible effect of alcohol in producing a negative is eliminated by a subsequent test a few days later. Venepuncture is a severe shock to many nervous cases, though it is still done for the sake of comparison. This test, supplemented with spinal puncture, may prove to give all the information obtainable.

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## FURTHER NOTES ON A MODIFICATION OF THE NOGUCHI TEST\*

BY NORMAN E. WILLIAMSON, M.D., STOCKTON, CALIF.

SINCE the complete inhibition of hemolysis should be the only criterion in titration of a syphilitic blood, the presence of demonstrable cells is all that is necessary for this purpose. Since no blood would be titrated which had not already shown complete inhibition in the standard quantity of blood mixture, 1 c.c., the titration may well begin with  $\frac{3}{4}$  c.c. of blood mixture and can be done according to the following table:

A blood I examined gave, when inactivated by the Noguchi system with the standard of 0.08 c.c., complete inhibition with 0.03 c.c. This would be equal to six plus. The same blood titrated by the method here given gave inhibition in the fourth tube which contained 0.0075 c.c. of blood or approximately 0.005 c.c. of serum. This would, according to this method, be 8 plus, so in comparing titration with the fresh blood with former methods, allowance should be made for this difference. It is readily seen in this case that by inactivation the blood had lost  $\frac{7}{8}$  of its strength in syphilitic reagin. If amboceptors capable of non-specific fixation lost as much, their action would, of course, not show. Dilution of fresh blood may serve the same purpose of preventing nonspecific fixation.

To save materials and also to get the effect of further dilution, one can use in the standard test, half the quantity of blood, or 0.06 c.c. in 3.94 c.c. salt solution, and use 1 c.c. for each tube. All materials can then be used in

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\*From the State Hospital, Stockton, Calif.



half strength and the results are excellent. It is well to pay no attention to minor degrees of inhibition of hemolysis.

For survey purposes of old patients in this hospital, I am using only the cholestrinized acetone insoluble fraction as antigen, repeating positives with the non-cholestrinized antigen. This saves material as old cases so far have not shown more than one positive in thirty patients.

Tube No.	Blood Mixture —0.12 c.c. blood; 3.88 c.c. salt solution with 4 parts per 10,000 sodium citrate	Antigen- Acetone insoluble frac- tion. 1 part: Salt Solution 9 parts	Comple- ment	Salt Solution	Shake and incubate $\frac{1}{2}$ hour in water bath at 37° C.	Ambo- ceptor 3 units to 1 c.c.	Shake and incubate 1 hour in water bath at 37° C.	Value of com- plete inhibition.
1	0.75 c.c.	0.1 c.c.	Units 2	.....		0.8 c.c.		3 plus
2	0.50 c.c.	0.1 c.c.	2	0.25 c.c.		0.6 c.c.		4 "
3	0.375 c.c.	0.1 c.c.	2	0.375 c.c.		0.6 c.c.		6 "
4	0.25 c.c.	0.1 c.c.	2	0.5 c.c.		0.5 c.c.		8 "
5	0.125 c.c.	0.1 c.c.	2	0.625 c.c.		0.5 c.c.		16 "
6	0.75 c.c.	.....	2	0.1 c.c.		0.8 c.c.		Anticomple- mentary control
7	0.75 c.c.	.....	.....	0.1 c.c. plus amt. of comp.		0.8 c.c.		Measure of human complement
8	0.5 c.c.	.....	.....	0.5 c.c.		.....		Control for cells

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## EDITORIALS

### *The Condition of the Circulatory and Respiratory Systems in Pneumonia*

VALUABLE information concerning the state of the heart muscle and of the vasomotor and respiratory centers secured from experiments on animals has been published at various periods during the past two years from the laboratory of Comparative Physiology of the Harvard Medical School. On account of the great importance which the conclusions so far reached must have in the investigation of the disease in man, the following brief review of the work is offered here.

The first paper, published by Porter and Newburgh<sup>1</sup>, deals with the state of the vasomotor center in rabbits, cats and dogs exhibiting marked symptoms of pneumonia as a result of infection with the pneumococcus of Fränkel or the streptococcus mucosus (pseudopneumococcus, pneumococcus mucosus). The first mentioned organism was injected intravenously in rabbits, and caused a septicemia, death occurring in from 48 to 96 hours. The pseudopneumococcus was introduced directly into the bronchi through a catheter in all three classes of animals; it caused acute bronchopneumonia, but some of the animals, particu-

larly the cats, ultimately recovered; the others, particularly the dogs, often in about twenty-four hours developed a comatose condition with a marked fall in rectal temperature. The condition of the vasomotor center in animals nearing death was ascertained by measuring the reflex change in blood pressure produced by stimulating depressor or pressor nerve fibers. It was found that the changes were of the same magnitude as those observed by the same methods of investigation in normal animals, thus proving beyond dispute that in animals about to die from pneumonia the vasomotor center is in a normal condition.

The next important paper of the series<sup>2</sup> deals with the condition of the heart muscle in dogs near death or just dead of pneumonia produced by intratracheal injection of *B. pneumoniae* (Friedländer). The condition of the heart muscle was determined by observing the work done by a piece of excised ventricular muscle perfused under pressure through the left coronary artery. The piece of muscle was connected with a muscle lever writing on a very slowly moving recording drum, the work done being gauged by the length of the period during which the muscle continued to contract and the total area of the contractions. To secure comparative figures the ingenious device was adopted of cutting out, from cards of uniform thickness, exact patterns of the tracings, which were then weighed. It is not generally known that Porter successfully used the above method for studying the beating of excised mammalian ventricular muscle just twenty years ago, which entitles him to the credit of being the first investigator to succeed in demonstrating that mammalian cardiac muscle maintains its rhythmical contractility outside the body.

Four series of experiments were performed, ten animals being used in each series. The general nature of the experiments and the average results are as follows:

PREPARATION	DURATION OF CONTRACTIONS	TOTAL WEIGHTS OF CONTRACTION AREAS
1. Normal ventricle and normal blood	181 minutes	8.84 gm.
2. Pneumonic ventricle and normal blood	187 minutes	8.46 gm.
3. Normal ventricle and pneumonic blood	70 minutes	3.40 gm.
4. Pneumonic ventricle and pneumonic blood	176 minutes	6.46 gm.

The results clearly demonstrate that in pneumonia the heart muscle is perfectly normal when it is fed either with normal blood (2) or with pneumonic blood (4), but that the efficiency of a normal heart is strikingly lowered when it is supplied with pneumonic blood (3). Two conclusions of the greatest consequence are thus permissible: the first, that the heart muscle is not functionally impaired in pneumonia; and the second, that this nonimpairment is due to the muscle having acquired some sort of immunity towards the poisonous influence which, as the results of the third series of experiments show, pneumonic blood exerts on normal cardiac muscle.

Having shown that neither the vasomotor center nor the heart muscle is affected in pneumonia, direction was centered on the behavior of the respiratory center.<sup>3</sup> The reason for choosing this as the next step to take in the investigation was the fact, often observed by the authors during the work described above, that the respirations frequently failed while the heart and vasomotor center were normal. The condition of the respiratory mechanism was, therefore, com-

pared in healthy dogs and cats with its condition in animals infected with pneumonia (*B. pneumoniae* of Friedländer, and, in one case, the pneumococcus).

The activity of the respiratory center was studied by observing the volume of air passing into and out of the lungs while the animal breathed through a tracheal cannula, and (Tissot) valves, into a graduated (Gad) spirometer and out of a bottle, the bottle and spirometer being also connected by tubing so as to form a closed system. The spirometer was caused to record its excursions on a drum. As the  $\text{CO}_2$  increased in the air of the system—determined by removing samples for analysis—the curve became deeper until the maximum reaction was reached. It was found that anesthetics materially depressed the reactivity of the center in normal animals, but that decerebration had no effect (after allowing the influence of the anesthesia necessary for the decerebration to pass off). The observations were, therefore, made on decerebrated animals except in the case of dogs in which the pneumonia had gone so far as to make them comatose. The average percentile increase in tidal air in normal decerebrated dogs as compared with pneumonic dogs *with the same initial ventilation* was found to be as follows:

CO <sub>2</sub> PER CENT IN AIR OF SYSTEM	PERCENTILE INCREASE IN C.C. AIR BREATHED.	
	Normal	Pneumonic
1	17	15
2	43	35
3	90	57
4	148	74
5	216	85

It was shown that the mechanical effect of the consolidation of the lungs was not responsible for these results; thus, they were not obtained after injecting starch into the bronchi, nor was the fall in excitability of the center in the pneumonia cases proportional to the degree of consolidation of the lungs. The degree of depression of the center was found to be proportional to the stage of the disease. By making a division into mild and severe cases, as determined by the behavior (lowering) of the rectal temperature, such results as the following were obtained:

REACTION TO 5 PER CENT CO <sub>2</sub>	PER CENT
In normal dogs (with varying initial ventilation).....	
In moderately ill dogs.....	127
In gravely ill dogs.....	68

Perhaps the most interesting of the papers is the last to be reported<sup>4</sup>, in which the possible causes for the depression of the respiratory center are investigated. Using the above described method for testing the excitability of the center, it was found that intravenous injection of the cultures of the *B. pneumoniae* into a normal dog did not cause depression, neither did injection of blood from another animal dying of the disease. These two observations show that the poisons produced in the blood by the growth of the organism are not the cause of the depression. The possibility next considered was that inadequate oxygen supply to the center might lower its excitability, the cyanosis so commonly observed as one of the symptoms of pneumonia giving some

weight to the suggestion. Experiments of various types were performed to test it; thus, pneumonic animals were supplied with an excess of oxygen by keeping them for long periods of time in a box ventilated with air containing several times the normal percentage of oxygen, but with entirely negative results, for neither was the progress of the disease checked, nor the condition of the animal materially improved, nor any change noticeable in the frequency or the depth of the respirations. Attempts to administer the oxygen by slowly injecting it into a capillary area (through the femoral artery) were disastrous, for the oxygen formed air emboli, which could be seen through the exposed femoral vein and which quickly killed the animal. These experiences do not offer much support to the clinical practice of oxygen administration in pneumonia. That oxygen starvation of the center could not be held accountable for the dyspnea was further shown by the inability to demonstrate a similar degree of dyspnea after reduction of the number of red blood corpuscles by hemorrhage or by combining some of the hemoglobin with carbon monoxide (by mixing illuminating gas with the inspired air).

It was also attempted to conserve the animal's strength and so prolong life or permit "the crisis" to be tided over by applying forced respiration by means of an artificial respiration apparatus, but again with entirely negative results; indeed it was found that the animals went on breathing at the usual (pneumonic) rate and depth in spite of the forced respirations. To make it perfectly certain that the depression of the center could not be due merely to its exhaustion as a result of the frequency and excessive intensity of its discharges, a condition of dyspnea was induced in normal animals by causing them to respire for several hours in an atmosphere containing an excess of carbon dioxide (with abundance of oxygen), and then removing them and, when the breathing had returned to normal, testing the response of their respiratory center by the above described method. It was found to be normal. Mere fatigue of the center cannot, therefore, be the cause of its depression in pneumonia.

In the face of so many negative results, it seemed as if no definite cause could be discovered to account for the dulling of the respiratory center, but a clue was secured by piecing together certain of the above observations; thus, that no dyspnea occurs when there is no inflammation of the lungs, although fatal bacteremia is present, as after intravenous injection of *B. pneumoniae*, and that such does occur when inflammation of the lungs is present, as when the bacillus is administered intratracheally, indicates clearly that it is not because of any toxic substance in the blood that the center becomes dulled, but rather that the inflammatory condition of the lungs of the same animal is the responsible factor. The question remains as to how the condition in the lungs comes to act on the respiratory center. Two pathways are open between the lungs and the center, the blood and the vagus nerves. The former is ruled out as concerned in connection with pneumonia, because blood from a pneumonic animal does not cause dyspnea when injected into one that is normal. By exclusion, then, it would appear that the association is affected through the vagus nerves, and it is certainly to be considered as one of the most interesting results

of recent research in experimental medicine that the authors should have succeeded in showing, by direct experiment, that this conclusion is the correct one. In short, they found that the excitability of the respiratory center to carbon dioxide—as measured by the above described method—is the same in vagotomized pneumonic dogs as in normal animals. In the vagotomized animals it was also observed that there is no dyspnea, and that the fall in the rectal temperature, usually observed shortly before death, was often absent, sudden death being, however, not infrequent.

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—J. J. R. M.

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### *Commercial Glucose As a Food*

WESENER and Teller<sup>1</sup> have recently reported a study of the chemical and digestive properties of commercial glucose. This term is applied to a product obtained by the action of certain catalytic agents upon refined starch, continued until the starch is converted into a mixture consisting principally of glucose, maltose and dextrins. The digested mass is purified by bone-black and then evaporated to a syrup. In this country cornstarch is used exclusively and the product is known as corn syrup. Wesener and Teller describe this preparation as follows: "Commercial glucose is a complex body of viscous consistency, running about 42 to 45° Beaumé, and containing 80 to 85 per cent of solids and 15 to 20 per cent of water. It is nearly water-white and possesses a mild sweet taste. The solids are composed almost wholly of sugars and dextrins, a minor portion consisting of a trace of mineral matter. The ash, present to the extent of mere traces, consists of mineral salts, including phosphates, sulphates, chlorides and carbonates, chiefly of sodium and lime. Tests for arsenic and other poisonous metals show these not to be present. Nitrogenous substances are present as mere traces, chiefly as protein bodies, amounting to about 0.06 per cent."

The sugars fermentable with baker's yeast are a mixture of maltose and dextrose, these varying in different preparations from 11.7 to 17.2 per cent of dextrose and from 22.9 to 16.4 per cent of maltose. There is a third substance which is less readily fermentable by ordinary baker's yeast but which is made fermentable by the action of certain enzymes, notably those present in pancreatin, Taka-diastase and malt, also by dilute hydrochloric acid under the influence of heat. This substance amounts to about 14 per cent of the glucose, when calculated as maltose, and about 8 per cent when calculated as dextrose. The unfermentable bodies after the removal of the substances above mentioned

consist of those bodies generally denominated as dextrins, and these after subjection to the hydrochloric action of diastase are converted into substances which under suitable conditions undergo almost complete alcoholic fermentation. Likewise, these dextrins after being acted upon by hot dilute acid become wholly fermentable. A good quality of Taka-diastase converts the unfermentable bodies into fermentable sugars, leaving only a small amount of unfermentable residue, which contains less than one per cent of the glucose taken. Pancreatin of good quality has a like effect, but in some instances the conversion was less complete than with Taka-diastase.

A comparative study was made of the action of hydrolytic agents, including enzymes, on commercial glucose and our chief carbohydrate foods, such as potatoes, breakfast cereals, bread, etc., and upon pure starches obtained from these foods. In these comparisons it was found that the carbohydrates of glucose agree closely in gas production with the carbohydrates of ordinary foods. When the latter are acted upon by isolated ferments and yeast they yield variable but appreciable amounts of unfermentable products.

Wesener and Teller conclude their report with the following statement: "The fact that commercial glucose, when it is treated with diastase and then subjected to yeast fermentation, is almost wholly converted into alcohol and carbon dioxide, goes to prove that it consists of products that are wholly assimilable, and, therefore, it furnishes a food to the body of a sugar nature. In this respect it is a more concentrated and at the same time a more readily assimilable food than are most of the carbohydrates belonging to the ordinary foodstuffs which first have to undergo cooking and then complete hydrolysis by the action of the digestive enzymes before they can be utilized by the body. In this respect glucose, pound for pound of dry weight, will furnish at least as much energy as does cane sugar."

Carlson, Hektoen, and LeCount<sup>2</sup> have studied the effects of commercial glucose when fed to white rats. Four groups of twenty-five each were fed on glucose bread; two groups of the same number each on cane sugar bread, and two groups on sugar-free bread. The feeding was continued through six months, a period of about one-sixth the average length of life in this animal. The quantity of glucose consumed each day by those fed on the glucose bread amounted to from 2.5 to 3.5 g. per kilo body-weight. "A parallel test on a group of persons weighing each 60 kilos and living sixty years on the average would mean a daily consumption of 150 to 200 g. commercial glucose per day for ten years." The animals were kept twenty-five in a cage and the ration to begin with for each cage consisted of 300 g. of bread, 150 g. of carrots, or of meat occasionally, and plenty of water. All debris was removed and a new supply of food given at a certain hour each day. As a rule the day's supply was wholly exhausted with the exception of the hardest bread crusts. After about one month the daily ration was increased, the same in each cage. The animals did not wholly escape infection and some succumbed to pneumonia and so-called rat-typhoid, but one group did not suffer from infection more than the others. Weighings were made every two weeks and the results showed that weight increased at practically the same rate in each group. In the course of

the experiment two rats of each group were injected intra-abdominally with 5 c.c. of a ten per cent suspension of sheep corpuscles per kilo of rat weight and six days later these animals were killed and the production of antibody determined. All groups corresponded in this particular and showed no difference from rats kept under usual laboratory conditions. The fecundity of the glucose-fed rats was slightly greater than that of the other groups.

These investigators state their conclusions as follows: "The addition of commercial glucose in the amounts of about 2.5 to 3.5 g. per kilo body-weight per day to the diet of white rats for a period of six months has no abnormal influence on the animals, either favorable or unfavorable, as determined by the rate of growth, fecundity, immunity reactions and the condition of the organs. As both the glucose-fed and the control groups were kept on a liberal diet throughout the observation period, the experiment does not show to what extent the commercial glucose was actually absorbed and oxidized, but in the quantities fed, the commercial glucose certainly has no injurious effects."

Sansum and Woodyatt<sup>3</sup> have used phlorhizinized dogs to determine to what extent the carbohydrates of commercial glucose are absorbable and convertible into d-glucose and consequently to what extent they are utilizable in the organism. "The idea suggested itself of passing a food through the body of a completely phlorhizinized dog and measuring its total yield of extra sugar in the urine, the well known phlorhizin technic being thus applied to a problem of food analysis. The extra sugar recovered would then naturally represent nothing which was either indigestible, unabsorbable or unassimilable."

With this idea these investigators fed completely phlorhizinized dogs with pure d-glucose and with commercial glucose and determined the extra sugar which appeared in the urine in each case. Theoretically all the d-glucose may be recovered in the urine, but practically in their experiments, only 74.6 per cent was so recovered, while 71.29 per cent of the commercial glucose was recovered. "On the basis that the sample of commercial glucose submitted for study contains 20 per cent of water, 100 g. of the solid residue is capable of affecting the urinary out-put of d-glucose from completely phlorhizinized and glycogen-free dogs in the same manner as though it contained 95.48 per cent of pure d-glucose. This implies that at least 95.48 per cent of the solid matter in the sample of commercial glucose studied is capable of passing through whatever physiological processes of digestion, absorption, and assimilation are necessary for its ultimate existence in the body as d-glucose."

"The animals showed no evidences of any deleterious effects attributable to the ingestion of commercial glucose, notwithstanding the large size of the doses (1 to 2 g. per kilo of body weight in three to six hours) and the great sensitiveness of completely diabetic animals to toxic influences."

One can only conclude from the evidence brought forward by the several investigators above quoted that Mendel<sup>4</sup> is justified in the following statement: "Corn syrups and glucose sugars artificially prepared from cheap sources of starch have survived the propaganda of prejudice and now represent one of the cheapest sources of wholesome nutriment."

There is nothing deleterious in commercial glucose as now prepared. It



consists of carbohydrates, which are in part predigested and in part partly digested. All of these are, after the completion of alimentary digestion, absorbable and assimilable and capable of supplying the body with energy. In short they comply with all the requirements of a healthy food. The average man needs in his daily ration about 540 g. of carbohydrates. It is best, indeed it is quite essential, that his daily ration should consist of mixed carbohydrates. If he took it all in the form of plain starch, his digestion would be overtaxed. If he took it all completely digested it would be absorbed so rapidly that the blood would be flooded with it and glycosuria would result. If he took it all in the form of cane sugar it would be too sweet and besides intestinal irritation would result. By thorough cooking of starchy foods man secures their partial digestion and their more ready absorption. Commercial glucose consists of partially digested carbohydrates and if a higher degree of sweetness is desired it may be mixed with cane sugar.

While the former is a manufactured product, the hydrolytic changes brought about in its preparation are identical with those occurring in the alimentary canal; consequently its predigested and partly digested constituents correspond to stages reached in the normal digestion of carbohydrates and supply the body with similar sources of energy. Commercial glucose may be regarded as a wholesome and nutritive food and may be mixed, as taste or fancy may dictate, with other carbohydrates in the preparation of the daily ration.

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<sup>3</sup>Sansum and Woodyatt: Jour. Biol. Chem., 1916, xxiv, 23.

<sup>4</sup>Mendel: Changes in the Food Supply and Their Relation to Nutrition, 1916, 18.

—I. C. F.

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### *Some New Facts Concerning the Adrenal Glands*

THERE is no subject in physiology that has attracted more attention in recent years than has the function of the adrenal glands. This interest has not been confined alone to the medical profession, for we find several semi-popular books in which attention is given to the relation which these glands bear to many of the functions of the human mechanism. The effects of the removal of the glands from the body are so drastic, and the physiological action of the substance, epinephrin, which they secrete, is so widespread and apparent that the importance of the glands cannot be questioned. Any new facts which add to our knowledge, or any experiments which disprove existing theories concerning the working of the glands are surely most welcome, for there is still much uncertainty as to the normal function of epinephrin and the conditions under which it is secreted into the blood.

Recent work has impressed one with the idea that the adrenal glands besides continually secreting some adrenalin into the blood, act, as it were, as

emergency auxiliary organs to the sympathetic nervous system, and in time of stress, pour out an increased amount of epinephrin into the blood. As examples and proof of the utility of such a mechanism, the effect that epinephrin has on increasing the amount of blood sugar is cited, and the occurrence of emotional hyperglycemia is attributed to the extra epinephrin which is poured out into the blood during great nervous excitement. Again, stimulation of the splanchnic nerves, emotional excitement, and injections of epinephrin all decrease the clotting time of blood, an obvious advantage to animals in combat. Morphine is known to reduce the amount of epinephrin which can be collected from the glands of a cat. This action is supposed to be due to the fact that morphine excites the cat and that this excitation brings about a depletion of the epinephrin in the gland by stimulation of the secretory nerves of the gland.

A recent paper by Stewart and Rogoff\* upon the influence of certain factors, especially emotional disturbances upon the epinephrin content of the adrenals, throws some new light upon the relationship of these glands to the central nervous system. These investigators could obtain no evidence that there is a decrease in the amount of epinephrin which can be obtained from the adrenal of the cat after prolonged emotional disturbance produced by frightening with a dog; nor could they obtain any evidence that the depletion of epinephrin in the adrenals following the administration of morphine is due to the attending nervous excitement. All the signs of morphine fright was observed in cats in which one adrenal was removed and the nerve supply of the other gland destroyed, thus establishing a condition which is known to protect the adrenal from morphine depletion. Moreover, dogs respond to morphine without excitement, and yet when given the alkaloid, marked depletion occurs in the epinephrin content of their adrenal glands. Evidently, therefore, excitement is not the cause of epinephrin depletion following morphine administration.

$\beta$ -tetrahydronaphthylamine administered to cats has been shown by Elliott to cause a marked depletion of the epinephrin store in one gland, the nerve supply of which is intact as compared to its fellow, the nerve supply to which has been previously cut. This effect he believed to be due to the emotional excitement which seems to accompany the injection of this drug in the cat. Stewart and Rogoff were able to confirm Elliott's experimental observation, but failed to obtain similar results with the rabbit, which apparently reacts to the drug as does the cat. In the case of the rabbit, both glands; i.e., the denervated and the normal gland, suffered some depletion of their epinephrin store. Evidently the action of  $\beta$ -tetrahydronaphthylamine on the adrenals in the rabbit differs from its action in the cat.

Another very important observation which these authors make concerns the reactions which occur in the denervated iris of the cat under conditions which have been thought to produce a hyperepinephrinemia. The denervated iris responds with dilation to very small doses of epinephrin, and this reaction has been used as a delicate test for epinephrin in the blood. Stewart and Rogoff were not able to demonstrate any difference in the character of the reactions

\*The Jour. of Exp. Med., 1916, xxiv, p. 709.

of the denervated iris elicited under conditions supposed to produce an outpouring of epinephrin in cats either normal or in which one adrenal had been removed and the nerve supply of the other gland destroyed some days previous to the experiment. By doing the experiment this way the otherwise fatal issue of double adrenalectomy is avoided.

Marked depletion of the epinephrin store of the innervated glands as compared with the denervated gland was observed in animals dead or dying from infections of various kinds. Stimulation of the splanchnics, a condition known to produce a hyperepinephrinemia, does not deplete the store of epinephrin in the gland except after very prolonged stimulation. Evidently splanchnic stimulation not only affects the discharge of adrenalin into the blood but also increases the rate of epinephrin formation.

We must conclude from this work that there is a difference in the type of response of the adrenals to conditions which deplete the store of epinephrin in the gland, and to those conditions which are supposed to be accompanied by an increased out-pouring of epinephrin into the blood. There is evidently one mechanism which controls the formation of epinephrin in the adrenals and another mechanism which conditions its secretion into the blood.

—R. G. P.

### *Epilepsy.*

A RECENT series of communications in current medical journals is concerned with the bacteriology of epilepsy, a subject first brought to attention by Bra in 1902, revived by Reed in 1914, and enlarged upon by the latter author in 1915 and 1916.

Reed has built his conceptions of the etiology of epilepsy upon the fact that constipation and epilepsy are associated in a large number of cases; and upon the theory that the constipation is the result of greater or less atony of the intestinal walls, especially those of the large intestine, or upon interference with the movements of the intestine because of abnormalities of position, or both. Evidently his whole point of view has been largely influenced by the opinions of Arbuthnot Lane. Once stasis has been produced, Reed believes that the body (the blood stream) is invaded by bacteria from the intestinal tract and these bacteria produce, by means of toxins, or by their presence in the central nervous system, the series of symptoms which we call idiopathic epilepsy. In proof of this theory of infection, he<sup>1</sup> and his assistant, Dr. Hyatt, report the very consistent finding of a peculiar organism in blood cultures which they say is identical with that originally cultivated by Bra, and which, from descriptions, is not clearly different from *Bacillus subtilis*. With pure cultures of this organism they are able to produce convulsive conditions in rabbits by introducing it into the ear veins.

Since Reed's reports, Wherry and Oliver,<sup>2</sup> Caro and Thom,<sup>3</sup> and Terhune,<sup>4</sup> have reported investigations aimed to discover more of the facts regarding the events associated with epilepsy.

The report of Wherry and Oliver is, as they acknowledge, a limited one. Nevertheless, working with Dr. Hyatt, they were unable, using the most modern methods, to discover any evidence of blood infection in four of Reed's cases. In a fifth case, one culture tube, in a series of ten, showed a growth which Reed reported contained *B. epilepticus*.

Caro and Thom reported their work with seventy cases from which 160 cultures were made with completely negative results except in four instances in which contaminations appeared. The contaminating organisms did not correspond with *B. epilepticus*. Moreover, Caro and Thom report that in 17 necropsies on epileptics, Canavan was unable to find any organism resembling *B. epilepticus*.

Terhune, on the other hand, reported that in his series of blood cultures from 24 cases of epilepsy he was able to isolate a bacillus, identical with Reed's, in 75 per cent of his cases. With this organism he was not able to cause convulsions in rabbits but was able to do so in cats.

Reed,<sup>5</sup> in a recent polemic, comments upon the work of those who have not been able to confirm his results, especially in the case of Wherry and Oliver, and says that "the assumption or inference that the organism does not exist is hardly tenable." No writer with whose work the reviewer is acquainted has said that the organism does not exist—they say they could not find it, when they didn't find it. Reed mentions confirmations of his work by various men at various points, but in no case are the details of their work known. As a matter of fact, the work that has been published on this subject from well organized laboratories in charge of well known workers concerning whose methods there can be no suspicion, is consistently negative.

This does not mean that the facts of Reed, Hyatt and Terhune are not true, but the fact that discrepancies are present makes it obvious that more systematic work should be done, preferably in large institutions supplied with every facility, and by thoroughly trained workers.

It is to be hoped that if the finally accumulated evidence is positive, the laboratory workers will not complain that the facts have been "beclouded by a policy of concerted and cumulative" affirmation.

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<sup>3</sup>Caro and Thom: Ibid., p. 1088.

<sup>4</sup>Terhune: Ibid., p. 1155.

<sup>5</sup>Reed: Jour. Amer. Med. Assn., 1916, lxvii, 1157.

—P. G. W.

### *The Factors Which Determine the Adequacy of a Vegetable Diet*

THE factors which determine the adequacy of a vegetable diet are of great interest especially now when we are hearing much of meatless, eggless, milkless, and butterless days. Moreover they are of vital concern to the medical man in the consideration of the so-called deficiency diseases. The vegetarian who religiously refuses all meat, eggs, milk, butter, etc., imposes upon

himself the necessity of wisely choosing from the staple vegetables a diet adequate to support growth and to maintain health. There can be no doubt that vegetable foods contain the factors essential for complete nutrition. The grazing animals grow entirely from food of plant origin. Nevertheless the vegetable foods ordinarily consumed by human beings lack several dietary factors, or contain them in inadequate amounts. It is, therefore, necessary to have some technical knowledge of food values if one wishes to choose a balanced vegetable diet. There is an accumulation of evidence to show that the seeds of any one of the common food plants do not maintain growth or support well-being when fed even in large variety. On the other hand by choosing proper proportions of a limited number of these seeds practically complete development may be obtained in the experimental animal. This observation shows that instinct, when the diet is confined only to the seeds of the common vegetable foods, is not a safe guide to complete nutrition.

McCollum and Davis<sup>1</sup> working with purified food substances found that besides protein, carbohydrates, fats and inorganic salts, two other substances were necessary. For these compounds they propose the terms, "Fat soluble A.," and "Water Soluble B." The former they find in great abundance in butter fat, and yolk of egg, and in fair amounts in meats and the leaves of forage plants. It is also present, although in inadequate amount, in many cereal seeds, corn apparently possessing a fair amount. The Water Soluble B. is present in many plants and is especially plentiful in the wheat embryo. McCollum and Davis believe that these factors are the same compounds which Funk has termed "Vitamins" and which this author believes include several dietary constituents of unknown chemical composition. They believe, however, that it is unnecessary to assume as Funk does the existence of more than the two factors above mentioned and are convinced that all the pathological phenomena can be accounted for by maladjustments relating to the following factors:—poor quality and inadequate quantity of the protein content; shortage of the substances A or B, or both of them; or the presence of some toxic substances in the natural food stuffs of inorganic or organic nature; or lack of inorganic salt.

McCollum, Simmons, and Pitz<sup>2</sup> in a recent article present data which they believe will at least serve to orient investigations on the question of nutrition by natural foodstuffs. They have tested fully three natural foodstuffs; viz., wheat, wheat embryo, and rice, upon a working hypothesis which postulates the need of but two chemically unidentified factors; i. e., A. and B. above described, in addition to the well-recognized constituents, proteins, carbohydrates, and inorganic salts. The results in the case of wheat are briefly shown in the following table; they offer an interpretation of the ways in which a food stuff may give rise to metabolic disturbance. The experiments were conducted upon different groups of young rats. Butter fat was fed to furnish the Fat Soluble A. substance. The embryo of the wheat contains sufficient Water Soluble B. substance. The protein was pure casein.

<sup>1</sup>Jour. Biol. Chem., 1916, xxiii, 231.

<sup>2</sup>Am. Jour. Physiol., 1916, xli, 333.

RATION.					RESULTS.
1.	Wheat kernel				Fails to support growth
2.	" "	+	salts		" " " "
3.	" "	+	" + protein		" " " " to maturity
4.	" "	+	butter fat		" " " "
5.	" "	+	" " + salts		Animals inactive, growth deficient
6.	" "	+	" " + protein		Fails to support growth
7.	" "	+	protein		" " " "
8.	" "	+	" + butter fat + salts		Supports growth and maintains well being.

The above results give a complete picture of the dietary deficiencies of the *wheat* kernel. Its proteins are of poor quality, it lacks the Fat Soluble A. substance, and has a deficient salt content.

Wheat *germ* was found to contain proteins of good quality, but to be deficient in salts and the Fat Soluble A. substance. Besides it was found to contain an oil which has a depressing effect upon the health and growth. The dietary deficiencies of polished rice were studied systematically as in the case of the wheat kernel. Rice was found to be lacking in proteins, inorganic salts, and in both the unknown chemical substances, A. and B. The existing data together with some experiments on the dietary factors present in corn, led these writers to conclude that the corn kernel contains proteins of poor quality, less than the required amount of the Fat Soluble A. substance, and an inadequate salt content. It contains a liberal supply of the Water Soluble B. substance.

From these experiments it is evident that the lack of a single factor will bring an animal into a pathological condition; or a toxic substance, such as that found in wheat embryo, will cause nutritive failure. The authors point out the fallacy of the assumption that the safest plan to assure perfect nutrition is to include a wide variety of foods in the selection of the constituents of the diet. Variety may make for safety, but the optimum result will not be obtained in a considerable number of cases. A knowledge of the specific properties of our natural food stuffs and their supplementary relations to each other will perhaps enable one eventually to compound simple and adequate diets of purely vegetable origin.

—R. G. P.

### *Carriers and Gall-Bladder Infections*

IN the group of diseases comprised of typhoid, cholera, and bacillary dysentery, one of the most important problems of preventive medicine seems to be the prevention and cure of gall-bladder and gall-passage infections. The prominence and importance of such infections in typhoid has been known for a long time; in the other instances it is less appreciated. Nevertheless several writers who have studied cases of semichronic intestinal carriers have shown that the carrier aspect was due to infection of the gall-bladder. In a few cases of bacillary dysentery the infecting organism has been recovered from the bile.

This subject of gall-bladder infection has been approached by Nichols\* from the experimental point of view. The experimental animal was the rabbit which was used because "in some respects, at least, the lesion in the rabbit is a counterpart of that in man, and it seems probable that, in working out means of preventing and curing carriers, the experimental lesion in the rabbit will be an important factor." Nichols' report deals with the mechanism of gall-bladder infections.

Nichols calls attention to the fact that in typhoid a gall-bladder lesion might be due to descending infection of the bile from the liver, to an ascending infection from the intestine, or to a transverse infection through the gall-bladder wall by way of the blood vessels. Previous to the time of Koch, the descending pathway was the generally accepted one, but since 1908 the transverse pathway has been that of choice. That ascending infection is not a common affair may be realized from the infrequency of colon infections of the gall-bladder. The same questions regarding the route of the infection arise in connection with cholera and dysentery.

As a result of his carefully planned experiments, Nichols arrives at the following conclusions: The theory of the production of gall-bladder lesions in typhoid by descending infection of the bile from the liver, receives support. More bacilli appear in the bile with increased doses and more gall-bladder infections are obtained by increased doses. More bacilli appear in the bile after mesenteric vein injection than after ear vein injection and more lesions result under the first condition. More bacilli appear in the bile after injection of the same dose in immunized animals than in normal animals and more lesions also result in immunized animals.

In cholera and dysentery the same mechanism is suggested with the additional factor of a portal system septicemia.

After the appearance of microorganisms in rabbit bile, their fate is apparently largely determined by the antiseptic properties of the bile. The antiseptic action is largely due to alkalinity. It is apparently possible to protect the rabbit to some degree against gall-bladder infections by a previous injection of sodium bicarbonate, and therefore alkali therapy is suggested in the prevention and cure of gall-bladder carriers.

—P. G. W.

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### *Ritual Murder*

THERE is a superstition prevalent among the more ignorant Christians of Southeastern Europe and Asia Minor that the Jews offer human sacrifices, and that they prefer young Christians, especially girls, for their victims. This superstition has a strong hold in certain localities, and is today a fixed belief held by many Syrian Christians, especially those living in Damascus. They bring this belief with them to this country, but, so far as we know, it has not led to any medico-legal investigation here. However, in Hungary and Bohemia it has led to criminal charges. One of the most celebrated cases of this

\*Nichols: Jour. Exper. Med., 1916, xxiv, 497.

kind is that known as the criminal process of Tisza-Eslar, which is a small community in Northeastern Hungary. On April 1, 1882, a servant girl, aged 14 years, 3 months and 23 days, was sent to the village to buy some colored yarns. It was found on inquiry that she made the purchase but did not return home. Indeed, she disappeared and the closest search failed to reveal any trace of her. On the day of her disappearance the Jews of the neighborhood held a religious ceremony at their temple in the village. The ignorant Christians began to suspect that the Jews had used the girl in their sacrificial rites, and about the end of April a little boy, aged 4 years and 9 months, a son of an attendant at the temple, reported that he had seen his people, the Jews, sacrifice the girl. At one time he said that she was bled from the thigh and another time that her throat was cut. The temple attendant and three other Jews were arrested and charged with the crime. Another son of the attendant, 13 years old, was questioned and at first denied any knowledge of the girl, but later he told that he had helped murder her. On the 18th of the following June a female body was found in the river below the village. She had on the clothes of the lost maid, and even carried on her arm a little bag containing the colored yarns which the girl had purchased the day of her disappearance, but the relatives and friends of the girl said that the body found in the river was not that of the lost girl. The head of the corpse found in the water was bald, and it was claimed that it had been shaven. The lost girl had done hard manual labor, and her hands were coarse, the skin thickened and callous, and the nails unkempt. The water corpse had beautiful white hands with tapering fingers and delicate nails. The local experts pronounced the corpse as that of a woman not less than 18, and most probably 20. It was claimed that the Jews had murdered the girl and had now gotten another body, probably by a second murder, had clothed this with the garments of the lost girl, and had thrown it into the river to lead the people to believe that the girl had been drowned. The local experts claimed that the body found had been in the water but a few days. At last some intelligent men were brought from the University of Koloszvar and others were called from Vienna and Budapest, and it was shown that the body was that of the lost girl, that it had been in the water so long that it had been partly changed into adipocere, and that the beautiful hands and tapering fingers were due to the fact that the epidermis had been removed by maceration, and that the scalp had been deprived of its adornment by the same process. It should be stated that the final examination was made in December, 1882, the body having been buried in dry sand from the previous June, when it was found in the water.

—I. C. V.

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### *Vitamins, Diet, and Beriberi*

BY the term "Vitamin" we have come to mean a chemical substance which is a constituent of certain foods, which in almost infinitesimal amounts has a beneficial influence upon the course of certain diseases, and which is an essential constituent of a normal diet. When such a substance, or substances, are eliminated from a diet, disease occurs. When it is added to an otherwise



vitamin-free diet the disease does not appear. When it is used therapeutically, the disease improves and health returns. The vitamins have been studied exhaustively especially by Funk and others, particularly in connection with the disease beriberi. It is with reference to this disease and its relations to vitamins that Vedder has contributed a recent interesting discussion.\*

From the time when Eijkman discovered that fowls develop a condition very closely resembling beriberi (polyneuritis gallinarum) if they are fed upon an exclusive diet of polished rice, interest in the so-called deficiency diseases has increased, and much energy has been expended upon experimental work designed to determine the means by which the pathologic effects are produced. It became plain, that many, at least, of the cases of beriberi studied were associated with the use of polished rice; i.e., grain which had been milled to the point where the whole pericarp had been lost, and only the polished carbohydrate kernel remained. It was later shown that persons might be fed upon polished rice with no evil effects provided a quantity of the husks removed by polishing were added to the ration, and also that unpolished rice did not conduce to disease. Still later, Fraser and Stanton showed that a protective substance might be prepared in solution from rice polishings by treating them with 0.3 per cent hydrochloric acid solution, and later Chamberlain and Vedder showed that the active substance was soluble in water and alcohol, but that it was insoluble in ether, that it was dialyzable, and that extracts of polishings containing only the water and alcohol soluble substances would cure infantile beriberi with marvelous rapidity. Then Funk obtained from rice polishings a crystalline base which would promptly cure fowls suffering from polyneuritis. It was for this substance that Funk coined the name *Vitamin*. Other workers studying this and allied substances of other origins, obtained materials which were different in composition from Funk's, but which were similar in pharmacologic action—hence the term, *Vitamins*. It then appeared that a pure crystalline *vitamin* had not been produced. Williams next attacked the subject from the synthetic standpoint and prepared substances which had decided antineuritic qualities.

It is obvious from the whole mass of evidence up to the present time, that these vitamins, whatever they may prove to be, are important factors in diets, which are essential in preserving the health of the individuals. Their absence is responsible for such deficiency diseases as beriberi, scurvy, and possibly pellagra.

Vedder says that the deficiency in the dietary that causes beriberi is not by any means limited to rice, for it has been shown repeatedly that too exclusive diets of ordinary white flour, canned goods which have been sterilized, and most carbohydrate foods, will produce polyneuritis in fowls, and beriberi in man. Rice, so far as is known, has not been shown to produce scurvy in man or animals, but with this exception practically all the "beriberi foods" have been associated with scurvy. Also it is noteworthy that where pellagra is prevalent in the United States, the sufferers from that disease are living very largely on articles that have been demonstrated to produce beriberi or scurvy, or both.

— P. G. H.

\*Vedder: Jour. Am. Med. Assn., 1916, lxxi, 1494.

*Medico-Legal Investigation of the Causes of Death*

THE writer has had an experience of many years as an expert in murder cases. This experience leads to the following statements, which may be of interest to physicians called upon to investigate the cause of death in suspected homicide.

Our criminal law fails to give directions concerning many points, and custom has apparently established no definite and satisfactory rules covering these matters. In my experience it has frequently happened that the initiative has been taken by friends or relatives of the deceased, sometimes by those financially interested, such as claimants to inheritance and insurance companies with which the deceased carried insurance; and in at least one celebrated case the initiative was due to the efforts of the man who finally was tried and convicted of the murder. It has not infrequently happened that officials permit some interested party to have full charge of the investigation of the cause of death, or to "work up the case." In most instances this has been due to the fact that the official, thinking that the suspicions were not well supported, has been unwilling to burden the county with the necessary expense, and has been ready to turn over the matter to anyone who would assume the financial responsibility of carrying out the investigation. In this way, it has happened that the obduction of the body and the toxicological examination have been wholly under the control of interested parties. While custom apparently permits this, it cannot be denied that such a procedure may subsequently give rise to much embarrassment in the prosecution of the case, and it certainly would be better for the proper officials to keep the investigations as to the causes of death under their own direction and to forbid participation in these investigations by all interested parties.

A few illustrations along the lines mentioned above may not be devoid of interest. In one instance, the behavior of the man whose wife had recently died gave occasion for much talk in the community, and the husband went to the prosecutor and asked that he be permitted to exhume his wife's body and have certain organs submitted to a toxicologist in order that he might be in a position to vindicate himself. This permission was granted. The husband, a physician, practically had charge of the investigation, and after the body had been exhumed and the stomach removed, he and an undersheriff took this organ to a toxicologist. The husband expressed some doubt as to which of two chemists he preferred and the stomach was left with one chemist with the request that nothing was to be done with it until further orders. A week later this chemist received a written order from the husband to deliver the stomach to the undersheriff who came alone this time. On the same day a stomach was placed in the hands of the second chemist, who examined it and reported it free from poison. However, it soon developed that the husband had obtained a stomach from the city physician of the place in which the second chemist resided. At this point the prosecutor saw that he must take the matter in hand himself. With a physician he went to the cemetery where the woman's body had been buried. Upon opening the grave the coffin was found to be empty. The body had disappeared. Search over the neighborhood failed to reveal any trace of it. With matters in this condition the husband was tried for the murder of his wife. A

shrewd lawyer, who defended him, declined to make any defense. He claimed that the prosecution had failed utterly. The only analysis which had been made had shown the entire absence of any poison. There was, therefore, the lawyer claimed, no evidence against his client. The judge permitted the jury to decide the case and the result was a split jury. Nearly a year later the body of the dead woman was found crowded into a barrel and hidden under a strawstack. The prosecutor was notified, but was never able to show who disposed of the body in this manner. The liver and other organs of the dead woman were examined by a third chemist and found to contain traces of arsenic. A second trial resulted in the conviction of the husband. Owing to certain technical errors, the Supreme Court ordered a new trial. This trial resulted in a split jury, and the husband who had stood three trials soon became insane and died in an asylum. It is not within my province to express an opinion concerning the guilt or innocence of the accused husband. One thing is quite certain, he was insane before he was tried at all. Whether he was insane or not at the time of his wife's death I cannot say. Through the bungling of the officials the county was saddled with the expense of three trials and the final result was no decision as to the man's guilt or innocence.

In the second case the prosecutor and a young physician, neither of whom knew anything about the proper procedure in such a case, resurrected the body from a country burial ground, took it to a shed nearby the village, and there, surrounded by men, women and children from the village, made the obduction. These officials had failed to provide themselves with receptacles for the tissues to be taken and examined, and the prosecutor asked one of the inhabitants of the village who kept a grocery store to bring from his store some fruit jars. The tissues selected for examination were put in these jars without having been even cleansed or even thoroughly inspected by the prosecutor or the physician making the obduction. Arsenic was found in large amount. When the case came to trial it was easy enough to show that the grocer who furnished the jars had not at all times been on friendly relations with the accused, and the suspicion that the grocer might have placed arsenic in the jars was brought into the case.

In the third case the body of the woman was found in the water. Her life was insured and the insurance company offered to relieve the county of any trouble or expense that might be involved in the investigation. The whole matter was turned over to the insurance company. Its agents made the obduction and its toxicologist made the chemical examination. The chemist reported the finding of morphine in the stomach. When the case came to trial the question really was not whether the accused killed his wife or not, but whether he killed her by the administration of morphine. Everything hinged upon the reliability and certainty of the tests employed by the chemist. This case resulted in a split jury and the accused husband was never tried again.

In still another case only the stomach was removed for examination. Some two years elapsed between the death and the trial. On the trial, evidence was introduced tending to show that the vomiting from which the woman suffered during the last days of her life was due to an extrauterine pregnancy. Inasmuch as the pelvic organs were not examined at the time of the obduction, this evidence could not be refuted, and again the case resulted in a mistrial.

In yet another case the stomach only was examined, two chemists failed to find any poison in the stomach. The prosecutor had a fixed idea that the man died from morphine poisoning and with the last remnant of the stomach he went to a chemist who thought that he found morphine. The case was tried and the wife of the dead man was convicted of murdering him. Later a new trial was ordered, and this time the body was exhumed and careful and thorough obduction made, and it was found that the skull had been fractured with an iron crow-bar. The wife had employed a negro to strike down her husband as he entered his home at night. Morally the woman was guilty, but the immediate cause of his death was someone else, and it was not a case of morphine poisoning.

In the legal investigation of the cause of death the medical man should make at the time accurate notes of everything he does and sees that may have a bearing upon the case. This should be done in the inspection of the body, while making the autopsy, and by the chemist, microscopist, or any scientific or medical man connected with the case. This record is known as the protocol, and this must be strictly a record of fact and absolutely without any statements of opinion. Indeed, it must be free from expressions of opinion. After the protocol has been finished the man who has made it may draw his conclusions from it, and these he may commit to writing, but on other sheets of paper. In my opinion both parties in the case should have copies of the protocol, but conclusions that are drawn from it are for the prosecutor alone as a rule, at least until stated by the witness on the stand. For instance, in making the obduction it would be the duty of the man making the section to note fractures of the skull, structural diseases of the heart or, in short, any abnormality, but it would be improper to state in the protocol that death resulted from this or that condition. It is proper for the chemist to state in his protocol that in the contents of the stomach or in so many grams of the liver he found so much arsenic, but it would not be proper to insert that this is enough to cause death. If the mucous membrane of the stomach shows erosions, statement to this effect should be made in the protocol, but a statement of an opinion as to the cause of these erosions would not be proper. In making an inspection of the dead body, which is sometimes technically called "view," all the surroundings should be observed and should be noted. A photographer may very properly be called into service here, but if so he must photograph things as they are and no rearrangement should be permitted. The conditions under which bodies are inspected are unlimited in variety, and the object should be to get a true picture either in written words or by the camera, or by both means. The environment should be closely scrutinized, footprints, evidence of a struggle, the presence of a weapon, the existence of powders or liquids that may contain the remnants of poison, and in fact everything should be seen and when seen their exact location with reference to the body should be recorded, and in doing this it is better to make exact measurements than to guess. If the body be clothed, the color, texture, arrangement, etc., of the dress should be recorded. The position of the body and its relation to other things may be matters of great importance. In one case in my experience it was found to be quite pertinent to determine whether the victim had vomited before or after he fell, and so slight a thing as a string of mucus extending from the dead lips to the vomited matter on the floor played quite a role in determining the truth of the story of the man who first reported

the murder and was finally convicted of the crime. The position of the hands of the deceased, are they closed, do they grasp anything, do they bear any marks of a struggle, are there stains on the face, hands or clothing of the deceased. All this and more must not be overlooked. Especially when the victim is a woman and attempted rape may have led to the murder, the underclothing should be carefully examined and seminal stains and bruises of the external genitals may be found. If there be wounds they should be carefully examined and their nature determined and recorded. The presence of letters or papers about the body or in the pockets should not be overlooked, and all such articles found should be turned over to the representative of the state. The question of identity is seldom a difficult one, but when not ascertained every mark that may bear on identification should be observed and recorded. The height should be accurately determined, the weight approximated, the color of the hair, beard and eyes noted. The absence of teeth or the presence of dental plates and fillings may be of service. When the face is badly disfigured by wounds an intimate friend may fail to identify a corpse either by direct inspection or by means of a photograph. In such cases, after the face has been photographed as found, the wounds may be stitched and the face brought as nearly as possible to its natural appearance and then photographed again. The presence or absence of rigor mortis, the state of preservation or anything else bearing upon the length of time that has elapsed since death are to be noted. After the body has been closely inspected in the exact position in which it has been found, it should be stripped of clothing and placed on a hard table or board and the entire surface including the openings into all cavities carefully examined. All marks, scars, wounds, and discolorations should be carefully scrutinized and proper notes made. Possible fractures and dislocations should not be overlooked, and any discolorations or excoriations about the neck deserve careful examination.

An obduction is an autopsy made for the purpose of determining the cause of death in the case of judicial inquiry. It differs from an ordinary autopsy, which applies to section of a body, known, or at least supposed to have died from known causes, in its purpose and may differ in the details with which it is carried out. An autopsy is usually for the purpose of determining the correctness of a diagnosis. As a rule, it is chiefly concerned in the examination of certain organs. It is usually made under the belief that a certain condition will be found. In other words, an autopsy is made for the purpose of confirming what is already more or less satisfactorily known to be the cause of death, or for the purpose of adding to our knowledge concerning the pathogenic changes that occur in the disease that caused death. On the other hand, an obduction is performed upon a body the cause of death of which is as a rule unknown. Moreover, and this is of great importance, the life of another may be directly dependable upon what is ascertained in the obduction. At least two medical men are needed to make an obduction. Both should be legally qualified and neither should have any personal interest in the case. They should be authorized to do the work by proper legal authority, and under such authorization they become for the time being officials legally qualified for the work in hand. It is not my purpose to give detailed direction for making an obduction, because the medical man who needs these directions should not be trusted with a procedure which is likely to

lead to criminal prosecution. It should be borne in mind by every man who is called upon to do this work that his record becomes an official document, that it will be criticised by men who are fighting for the life of a client, and nothing short of honest, thoroughly scientific procedure on his part will stand the test. It is no uncommon occurrence to see men who have made obductions greatly humiliated in trials for murder, especially for errors of omission. Often this is due to lack of experience in the legal officer who orders a partial examination, or orders one done under conditions of time and place that render thoroughness impossible, and the same legal official when he finds his case placed in jeopardy by the incompleteness of the obduction, is quite willing that the medical man who has carried out his orders should bear the blame. A medical man should absolutely refuse to make an obduction unless the conditions are the best that can possibly be obtained. However, the conditions are sometimes such that a perfectly satisfactory obduction cannot be made. The body may not be found until decomposition is far advanced, or it may be greatly mutilated, or suspicion of homicide may not arise until months and possibly years after death, or the body may have been frozen or alternately frozen and thawed several times, or, and this happens frequently, the obduction may not be held until after the undertaker has pumped the cavities and possibly the blood vessels as well, full of some fluid. For such conditions the legal official is not responsible and the medical man will have to do, as he is compelled to do in many other instances, the best he can and take whatever criticism may come. When possible the obduction should be held within forty-eight hours after death. If possible it should be done by daylight and in private. It should be complete. The calvarium should be removed, the thoracic and abdominal cavities opened by an incision extending from the chin to the pubes. All the cavities should be opened and the organs inspected *in situ* before any of them are removed. In dissecting the scalp, injuries to the skull should be sought. After the removal of the calvarium the meninges and then the surface of the brain, so far as it can be seen, should be examined. After all the organs have been examined *in situ* the brain should be turned out and every portion of the surface examined and then this organ should be dissected, careful search for hemorrhage being made. The dissected brain should be placed in a proper receptacle and preserved for chemical examination and microscopical study. After the pleural and thoracic cavities have been examined and ligatures passed about the larger vessels, the heart should be removed, the cavities opened, the valves examined and the organ placed in a proper receptacle. If there be any suspicion of pathological change in the lung a portion should be removed for closer study and microscopical examination. The stomach with proper ligatures around the esophagus and duodenum should be separated, lifted out and opened over a clean receptacle. After careful inspection of every part of the stomach, it and its contents should be placed in a jar. The small intestine should be removed and the whole length of the intestine examined both outside and inside. The small intestine should be preserved. The liver, kidneys, pancreas and spleen should be examined and portions of each preserved in separate jars. It is best to remove and preserve both kidneys. With females the whole internal genitalia should be removed, examined and preserved. In no case should two organs or portions of two be placed in the same receptacle.

It should never be forgotten in holding an obduction that the object is not only to find the cause of death ; but to exclude every other possible cause. Failure to recognize this has led to the miscarriage of many a case of trial for homicide. The prosecutor learns that Mr. Blank is suspected of having poisoned his wife who died a week ago. He investigates and finally orders the body disinterred ; the stomach only is removed and sent to a toxicologist. Arsenic is found and Blank is arrested and months later the trial is on. Now, the prosecutor learns that a positive diagnosis of arsenic poisoning cannot be made from the symptoms, that Mrs. Blank suffered for years from a combination of uterine and kidney diseases, that frequently she suffered from profuse vomiting followed by collapse and that many times it was thought that she would die ; that her kidneys undoubtedly were destroyed by disease, and that her ovaries had been masses of corruption for years, and the small amount of arsenic found by the toxicologist was due to the bismuth subnitrate, untold quantities of which this woman had swallowed. The prosecutor, thoroughly convinced of the guilt of the accused, feels that the case is slipping through his hands because someone has blundered, and wreaks his vengeance on the poor medical man who made the obduction and did not have sense enough to look after the kidneys and ovaries. Honest, if not learned, experts testify that every symptom that Mrs. Blank is said to have exhibited may have been due to chronic ovarian irritation combined with Bright's disease. A reasonable doubt arises in the mind of some juror and there is a disagreement. The trial has cost the county thousands of dollars and there is much grumbling among the taxpayers. Possibly the prosecutor is encouraged, persuades the board of supervisors to employ eminent legal help and the case is tried again, but most likely the same result follows. This is not an overdrawn statement, and its actual occurrence is not infrequent in the annals of criminal law. Much time and labor have been lost ; much money has been expended and justice has wholly miscarried simply because the obduction was not properly conducted.

Concerning the organs to be submitted to the toxicologist, there are two widely adopted customs that should be abolished because they frequently lead to perplexities the solution of which may be well nigh impossible. First, it is generally assumed that in suspected poisoning the stomach only, or at most the stomach and the duodenum, is needed by the chemist. The chemist needs, in addition to the stomach and duodenum, a portion of the liver, one kidney, the urine if there be any in the bladder at the time of the obduction, the heart or half of this organ and a portion of the brain. In exceptional instances he may need portions of other tissues, but those mentioned above should be furnished him in every case. Each organ or part should be placed in a jar by itself. That this advice is important the following statement of the happenings in a case may be mentioned :

The stomach and rectum were placed in one jar and in these tissues together the chemist found an amount of arsenic which he estimated for the entire content of the jar to be as much as 20 grains, but he could not say how it should be apportioned to the organs. All the arsenic might have been, when the tissues were removed from the body, in the one or in the other. The defense claimed that a large amount of white arsenic had been suspended in water and injected

into the rectum a few hours after death. It will be seen from this that the advice to place each organ or part in a receptacle of its own is not due to a whim, but is founded upon practical experience.

The use of some preservative for the tissue removed for examination is sometimes necessary. Fortunately within recent years formaldehyde has become the basis of embalming fluids, the best of which consists of an 8 to 10 per cent solution of this substance with from 2 to 5 per cent of potassium nitrate. These are the essential ingredients of Kaiserling's solution and for both the toxicologist and the pathologist this can hardly be improved. When the body has been well embalmed with the above mentioned fluid, the addition of a preservative to the removed organs is not necessary. If too dry a small quantity of embalming fluid may be added.

The second mistake, which is well nigh universally practiced and which should be continued no longer, is founded upon the theory that in cases of suspected poisoning a chemical examination only is desirable. In every case of suspected poisoning, portions of the walls of the stomach, liver, kidney, heart and brain, and in the female, of the ovaries and uterus should be properly hardened, sectioned, examined microscopically and preserved as exhibits. The proper carrying out of these procedures would prevent many complications and positively dispose of many doubts that arise in trials for murder by the administration of poison. As has been stated a frequent defense in these cases is that the deceased came to his death from natural causes and this claim would be easily disposed of if microscopical preparations from the organs above mentioned were made. Moreover, the object of the prosecutor is not to convict; it is to see that justice is done and this can be secured with certainty only after a most exhaustive search for the cause of death. Some poisons induce highly characteristic histological changes and the finding of these would furnish valuable confirmatory evidence in case the presence of the poison is obvious by chemical tests. Again, the deceased may have had a diseased heart, liver, kidney, or other organ, and yet death may have been due to the administration of poison. In such instance it would certainly be well for the prosecutor to know the facts. Not only is the histological examination of tissues neglected in cases of suspected murder by the administration of poison, but in many such cases the gross examination of all the parts of the body is neglected. This is a way of opening up a sure and safe escape for the accused.

The expert in examining into a gunshot injury should be alert, and carefully observe all the surroundings at the place where the wound was inflicted. Photographs are desirable and their veracity cannot be questioned. The position of the body of the wounded man at the time he received the shot should be determined. His clothes should be examined for holes, burnt spots and stains. The position of the assailant may in some cases be determined quite positively or facts that show that the accused's account of the injury cannot possibly be true may be brought to light; or on the other hand the accused's claim may be substantiated.

There is no law and apparently no common custom governing the transfer of portions of the body or other material, suspected to contain poison criminally introduced, to the toxicologist.



A chemist who has done much toxicological work could keep busy and get himself into all kinds of trouble if he would listen to all the requests made to him. He receives frequent letters from persons in many walks of life, telling of suspicions of attempted poisoning and asking him to make analyses. A wife suspects that her husband has put some deadly drug in her food; she wants her suspicion kept profoundly secret. She would not hint it to anyone, but she would be under many obligations to the chemist if he would make an analysis and set her mind at rest. Other requests are more authoritative: A physician thinks someone, usually husband or wife, is administering poison to his patient by putting it in his medicine. There is only one proper answer to make to all these requests whatever their source and it is this: "If you suspect that someone is using or attempting to use poison in a criminal way, report your suspicion to the prosecutor of your county and deliver any suspected material you may have into his hands. I can have nothing to do with any suspected material unless I receive it through official channels." It is the duty of the prosecuting attorney to inquire into the commission of crime and the toxicologist had better leave these matters to the official.

Usually the material for toxicological examination placed in clean fruit jars, only one organ or part of organ in one jar, is sealed by the official in charge of the case, prosecutor or assistant, sheriff or assistant, or by the medical men who have made the autopsy, and carried or sent by express to the toxicologist. Any of these methods of transmission seem to be satisfactory to the courts providing the material can be traced and the possessor at every stage identified. Usually in the course of the trial the medical man who made the obduction testifies that he removed such organs and placed them in jars, which to his knowledge must have been free from poison, sealed them (describing the seal) and delivered them to some official (prosecutor or sheriff usually or an assistant to one of these). Then the official testifies that he received them (seals unbroken) and transferred them to the toxicologist either in person or through the express company.

A protest should be made against the usual method of sealing jars containing material for the toxicologist. Fruit jars are suitable and are usually selected and the cap is covered with a mass of sealing wax. This is crude, not distinctive and is removed with difficulty. After the material is placed in the jar, the cap should be screwed on and the whole jar should be wrapped in heavy paper after which a tape string should be passed around the jar both longitudinally and horizontally. The distinctive seal should be placed on the points where the longitudinal and horizontal tapes and strings cross.

When the chemist receives the material directly from an official or his representative he should give a receipt in which he should specify the number and kind of jars and any labels that they may carry. He should not say in this receipt "I have this day at 10 A. M., received from John Jones, Sheriff of Ralls County, the stomach of Jane Potter," but he might say "I have this day at 10 A. M., received from John Jones, Sheriff of Ralls County, a quart Mason fruit jar said to contain the stomach of Jane Potter." On receipt of tissue for toxicological examination the chemist should place the material under seal and so keep it that he can testify that no one, save himself, has had access to it and

when the trial is on he should be able to produce all receptacles in which the organs were brought to him with such parts that have not been destroyed in making the chemical analysis. The toxicologist should keep a seal on his lips as well as on his private work room or his poison case. Troublesome inquiries should be referred to the attorney in charge of the case and this is usually the prosecutor; however, there are instances in which toxicological work is done for the defense, and very rarely the trial judge orders an examination made by independent parties for himself. We have known of this having been done in one instance and after receiving the report of the toxicologist the trial judge dismissed the case, although the chemist employed by the prosecution had reported the presence of a poison. This case was not brought to a hearing before a superior court and there is, so far as we know, no ruling on this point.

The question of the amount of poison recovered by the chemist from the tissue is a constantly recurring one in trials for homicide by this means. The courts of all civilized countries have generally held that it is not necessary for the chemist to show that there is a fatal dose in any organ or even in the whole cadaver. If poison be found and the jury be convinced that the accused administered it, this is generally held to be sufficient to convict. Homicide by the administration of poison is always premeditated murder. This of course, does not include accidental poisoning in which a poisonous substance is administered by mistake when something else was intended. If it can be shown that John Jones administered a poison to his wife knowing or believing that it was a poison, it is assumed that he gave it with ill intent. He cannot claim, for instance, that he was giving her arsenic as a tonic or to improve her complexion and that she died from some other cause and that the arsenic found in her body by the chemist is thus accounted for.

If the chemist can isolate the poison sufficiently pure to determine the amount this should be done. Moreover, he should if possible preserve portions of the poison and visible tests, to serve as exhibits. This can usually be done with the inorganic poisons, but exhibits of the poison cannot be demanded with many of the more deadly organic poisons. Rarely crystals of strychnin, morphin, chloral and other organic poisons can be obtained not only in quantity sufficient for all the necessary test, but some may remain for exhibits.

—V. C. V.

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## ORIGINAL ARTICLES

### FURTHER STUDY OF THE HISTOPATHOLOGY OF THE AUTONOMIC NERVOUS SYSTEM IN GOITER\*

BY LOUIS B. WILSON, M.D., ROCHESTER, MINN.

WITH A NOTE ON THE SPECIAL TECHNIC OF RECONSTRUCTIONS AT  
HIGH MAGNIFICATIONS.

BY THOMAS O. YOUNG, B.S., MINNEAPOLIS, MINN.

MANY of the older clinical writers classified exophthalmic goiter as a disease of the sympathetic nervous system. It is only in recent years that the primary importance of the thyroid has become recognized. Although we must give full weight to overfunction of the thyroid as the direct cause of hypermetabolism, which is the dominant factor in the syndrome designated "exophthalmic goiter," yet it must be confessed that when we attempt to explain the primary cause of overfunction of the thyroid, all our hypotheses are unsatisfactory.

Cannon<sup>1</sup> has shown that by the constant stimulation of the thyroid produced by anastomosing the phrenic nerve with fibers of the cervical sympathetic, he has been able to produce many of the symptoms of hyperthyroidism. McCarri-son<sup>2</sup> has adduced a large mass of data, both clinical and experimental, supporting the hypothesis that both simple and exophthalmic goiter are due to water-borne infection. However, the theory that the thyroid is excited beyond its normal function by infection within the gland has received little supporting evidence in the bacteriologic findings, in the histologic appearance of local inflammation, or in the clinical course of the disease.

Some time ago in an examination of the changes in the various skeletal muscles in cases of exophthalmic goiter, I was struck by the extremely erratic distribution of the degeneration in the various muscle fibers and bands. Such

\*Presented before the Minnesota State Medical Association, Minneapolis, Oct. 12, 1916.

an erratic distribution is difficult to explain on any theory of work-change or of the irregular vascular distribution of a blood-borne toxin. On the other hand, it might readily arise from the irregular destruction of selected neurons, or portions thereof, controlling various muscle bundles. In consideration of these facts from the clinical, experimental, bacteriologic, and histologic standpoints I was led to investigate more carefully the histopathology of the autonomic nervous system in relation to goiter.

#### PREVIOUS REPORTS IN THE PRESENT SERIES OF OBSERVATIONS.

Durante and I<sup>3</sup> have recently reported our observations on twenty superior cervical sympathetic ganglia removed at operation from sixteen patients with hyperplastic toxic (exophthalmic) goiter. Our findings may be briefly summarized as follows:

1. Definite histologic changes in the cells of the cervical sympathetic ganglia in hyperplastic toxic (exophthalmic) goiter occurred in all cases examined.
2. These histologic changes consisted of various stages of degeneration: namely, (a) hyperchromatization, (b) hyperpigmentation, (c) chromatolysis, and (d) atrophy or (e) granular degeneration of the nerve cells.
3. Some of the ganglia contained cells resembling the partially differentiated cells found in the ganglia of infants.
4. Accompanying the more advanced changes in the ganglion cells were similar degenerative changes in the nerve fibers and an increase of connective tissue throughout the ganglion, but especially in the outer and middle coats of the vessels and in the periganglionic tissue.
5. So far as could be determined from the small number of observations, the pathologic changes in the cervical sympathetic ganglia were parallel to the stage and intensity of the symptoms of hyperthyroidism and to the hyperplastic and regressive changes in the thyroid.

In addition to the above, I have recently reported<sup>4</sup> my observations on a study of the cervical sympathetic ganglia removed at autopsy from twelve patients dying during the course of exophthalmic goiter, the observations on which were controlled by similar studies on sympathetic ganglia removed at autopsy from patients dying of diseases other than exophthalmic goiter and by studies of Gasserian ganglia removed at operation from patients with trifacial neuralgia. The results of these latter studies show that the changes in the ganglia cases of exophthalmic goiter coming to autopsy are parallel in all respects with those in the ganglia removed at operation, while the ganglia removed from patients coming to autopsy from other diseases and the ganglia removed at operation for conditions other than exophthalmic goiter furnish valuable negative controls. In the latter report I have also included a brief preliminary note of experimental work in the production of the degenerative changes in the ganglionic cells. In one animal (goat) lesions parallel with those in the cervical ganglia removed from patients with acute exophthalmic goiter were produced by the injection into the capsule of the ganglion of a virulent culture of *Bacillus bronchisepticus*, the bacillus associated with canine distemper. This experimental work is still in progress, and will be made the subject of a subsequent report.

## SUBJECT MATTER OF PRESENT STUDY.

I wish to present more in detail and from a different viewpoint the general character of the histologic lesions. The study of these turns on the irregularity of their distribution, their extent, and the probable order of the various phases of degeneration.

When one studies the pathologic changes in isolated sections of the ganglia, it is difficult to determine the exact distribution of affected cells, though even in studying nonserial sections he is struck by the presence of apparently perfectly normal cells in immediate apposition to others that appear to be in advanced stages of degeneration. Since, however, the ganglion cells are of much greater diameter than the thickness of good paraffin sections, it is possible to interpret the apparent irregularities in size and irregular distribution of pigment in the ganglionic cells in a single section on the theory that they are the result of oblique cuts including only small portions of cells. In order, therefore, to determine the extent of the destruction of the cells one must study series of sections at least of sufficient extent to include all portions of a group of ganglionic cells selected in a given microscopic field in a section near the middle of the series. All preceding and succeeding sections in which these individual cells appear must be studied. I recognized this principle early in the work and all material has been studied in such serial preparations. One is able thus to demonstrate beyond a doubt the character and extent of the changes in a given group of ganglion cells.

This summer, Mr. T. O. Young, a medical student in the University of Minnesota, under my direction has prepared a number of Born wax reconstructions of groups of cells selected as above indicated. A description of his technic is herewith appended.

All the reconstructions were made at a magnification of 600 diameters, and photographed down to a magnification of 300 diameters, at which magnification they are herewith reproduced. In examining them the fact must not be lost sight of that they represent groups of cells selected as above indicated: i.e., those of each of which some portion appears in a single section of the series; thus while all portions of each cell in the group is shown, the group itself covers the distribution of the cells in 2 dimensions only. The interior details of the cells can, of course, be represented in the opaque models only diagrammatically and by arbitrary signs. With these, however, and the knowledge that the relative shape and size of the cells has been preserved, the analysis is interesting.

## PROTOCOLS.

*Case I.*—A-153535, Fig. 1. (For previously reported details of this case see Case II in article Ref. 4.) This reconstruction is from a group of ganglion cells in the left superior cervical sympathetic ganglion of a female 19 years of age who had had severe symptoms of exophthalmic goiter for five months. Of the nine cells shown, four, A, B, C, and D, are apparently normal and five, E, F, G, H and I are pigmented. In one, I, the most pigmented, the nucleus has disappeared; in the other eight the nuclei show no changes of sufficient importance to warrant our designating them as degenerated.

Fig. 2. This reconstruction is from another area in the same ganglion as that shown in Fig. 1. In this area, three cells, A, B, and C, are apparently normal and four, D, E, F, and G, are pigmented. In two, D and E, the nuclei

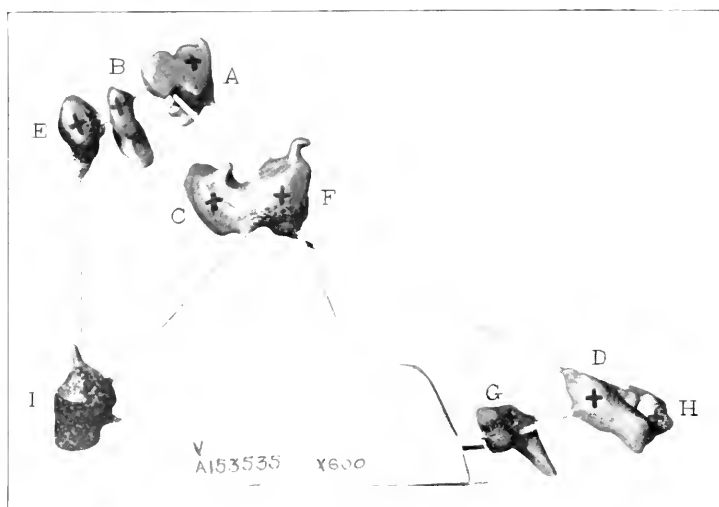


Fig. 1.

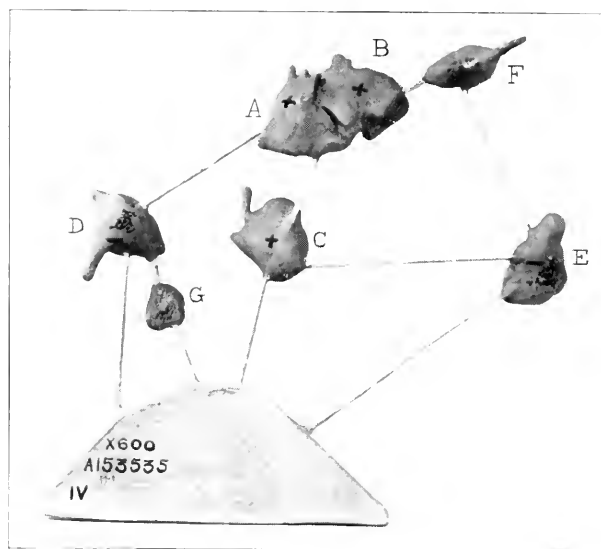


Fig. 2.

are in an advanced stage of degeneration; in the other two, F and G, they have completely disappeared.

*Case II.*—A-165975, Fig. 3. This reconstruction is from a group of ganglion cells in the right superior cervical sympathetic ganglion of a male 30 years of age who had had severe symptoms of exophthalmic goiter for three months. His left superior thyroid vessels had been ligated nine days and his



Fig. 3.

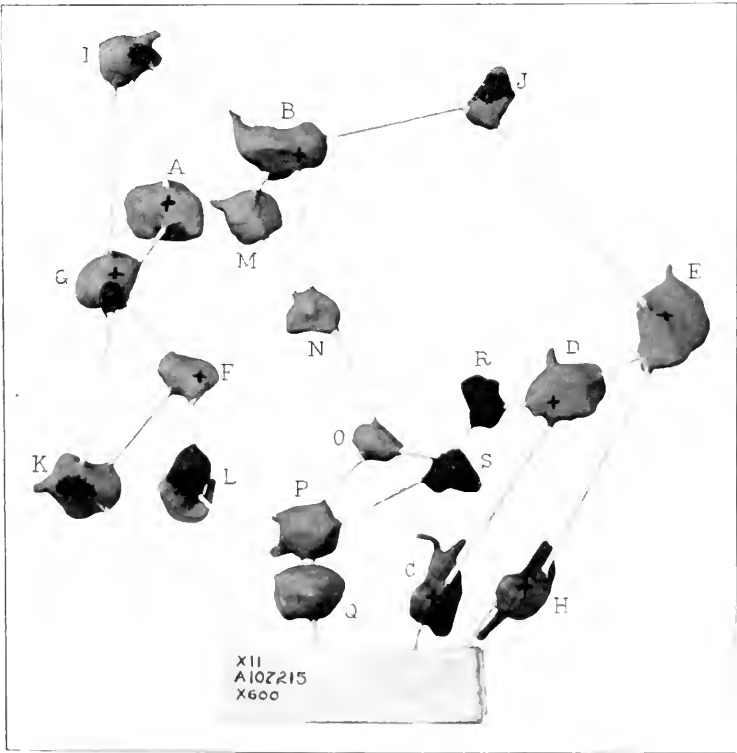


Fig. 4.

right superior thyroid vessels four days before his death, the immediate cause of which was a bilateral disseminated bronchopneumonia. His myocardium showed very marked fatty changes. Of the eleven cells shown, four, A, B, C and D, are apparently normal, both in their cytoplasm and nuclei. One, E, is atrophied and the nucleus is in an advanced stage of degeneration but without pigmentation in the cytoplasm. In two, F and G, there is advanced destruction of the nuclei, atrophy and advanced pigmentation. In two, H and I, there is atrophy and complete destruction of the nuclei, but no pigmentation. In one, J, there is some atrophy, advanced pigmentation and complete destruc-

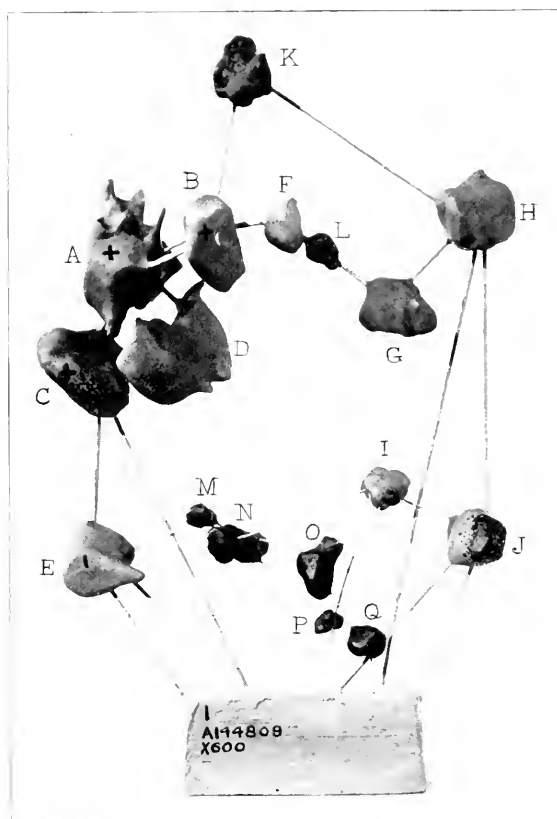


Fig. 5.

tion of the nucleus. Of one, K, only a mass of pigment about  $1/8$  the normal size of the cell remains.

*Case III.*—A-107215, Fig. 4. (Case I, Ref. 3.) This reconstruction is from a group of ganglion cells in the left superior cervical sympathetic ganglion of a female 24 years of age who had had symptoms of severe exophthalmic goiter for four months. Of the nineteen cells shown, five, A, B, C, D, and E, are apparently normal in size, character of protoplasm, and the nuclei. One other, F, may be normal, although it is quite small. Two, G and H, are heavily pigmented but show nuclei which are apparently normal. Four, I, J, K, and L, are heavily pigmented and show no nuclei. Five, M, N, O, P, and Q,



are atrophic, show no pigment but are without nuclei. Two, R and S, are reduced to relatively small masses of pigment only.

*Case IV.*—A-144809, Fig. 5. (Case III, Ref. 4.) This reconstruction is from a group of ganglion cells of the left superior cervical sympathetic ganglion of a female 30 years of age who had had severe exophthalmic goiter for eight months. Of the seventeen cells shown, two, A and B, show apparently normal protoplasm and nuclei though they are hypertrophied. One other, C, near them is markedly hypertrophied and though its nucleus is normal it shows beginning pigmentation. Another, D, in the immediate vicinity is markedly hypertrophied, shows beginning pigmentation and its nucleus has completely disappeared. One, E, which is normal in size shows beginning degeneration of the nucleus and no pigmentation. Three others, F, G, and H, though showing no pigmentation, show complete destruction of the nucleus. Two, I and J,

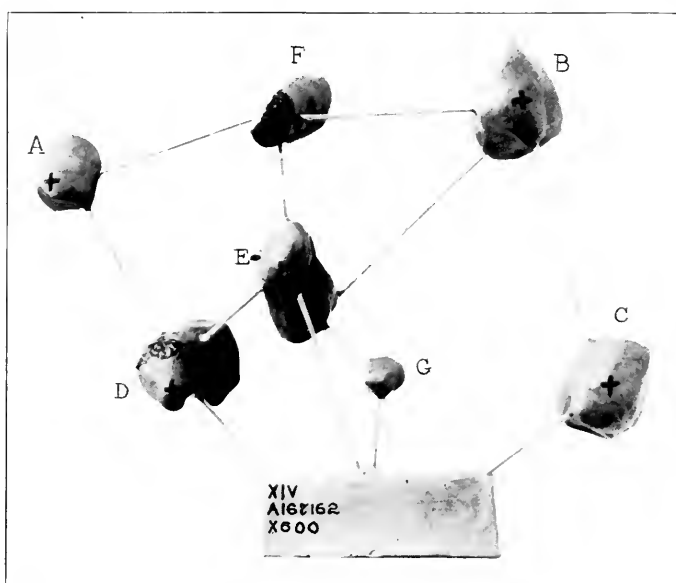


Fig. 6.

show advanced pigmentation with destruction of the nucleus, and seven, K, L, M, N, O, P, and Q, are reduced to atrophic masses of solid pigment.

*Case V.*—A-167162, Fig. 6. This reconstruction is from a group of ganglion cells in the right superior cervical sympathetic ganglion of a female 24 years of age who had had very severe symptoms of exophthalmic goiter for 3 months, remission of symptoms for 4 months, a severe relapse for 2 months, and then almost complete remission of all symptoms except severe exophthalmos during the last 6 months preceding operation (sympathectomy). Throughout the sections from this case hypertrophy of the ganglion cells was marked. In the group shown, one cell, A, is apparently normal in size, composition of its protoplasm, and character of its nucleus. Two, B and C, are very markedly hypertrophied, but with nuclei which show no degenerative changes. One, D, is hypertrophied and pigmented but with an apparently normal nucleus. Another, E,

is hypertrophied with an apparently normal nucleus. Another, F, is pigmented and shows no nucleus. Another, G, is atrophied, nonpigmented and without a nucleus.

*Case VI.*—A-129966, Fig. 7. (Case II, Ref. 3.) This reconstruction is from a group of ganglion cells in the left superior cervical sympathetic ganglion of a female 30 years of age who had had severe symptoms of exophthalmic goiter for a year. An examination of the sections from this ganglion showed in most areas a marked atrophy of the ganglion cells. The group selected for reconstruction is from an area in which the cells are hypertrophied. In the model each cell is bisected and on the cut surface are indicated the pigmen-

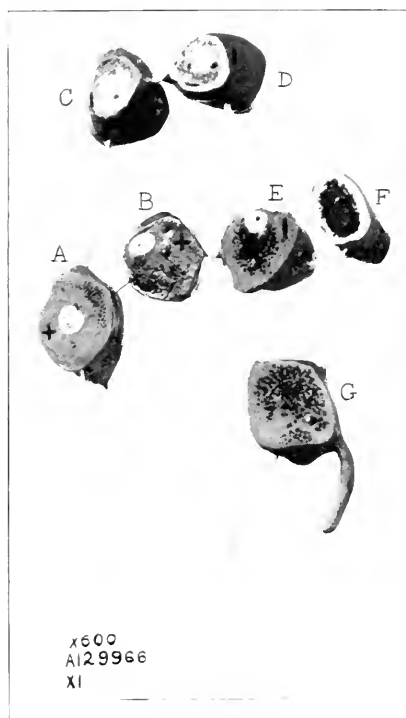


Fig. 7.

tion and appearance of nuclei; the latter is also indicated by the arbitrary signs. A photograph has been taken of the model with the loose portion of each cell removed. Of the seven cells shown, none are normal though two, A and B, show nuclei without material degeneration. In three others, C, D and E, degeneration has begun in the nuclei, and in two, F and G, the nuclei have completely disappeared. All the cells are markedly pigmented.

*Case VII.*—A-136418, Fig. 8. (Case IV, Ref. 4.) This reconstruction is from a group of ganglion cells in the superior cervical sympathetic ganglion of a female 25 years of age who had had moderate symptoms of exophthalmic goiter for one year and eight months, a period of exacerbation lasting three weeks and then partial relief by double ligation of the thyroid vessels fol-

lowed 4½ months later by thyroidectomy. At autopsy nine days after operation the cervical sympathetic ganglia were noticeably enlarged. Of the thirteen cells shown, two, A and B, are apparently normal. Three others, C, D, and E, show normal nuclei but are stained by diffuse pigmentation. Three others, F, G, and H, show normal nuclei, diffuse pigmentation, and in addition, masses of heavy pigmentation. One, I, shows diffuse light pigmentation and a partially degenerated nucleus. Two, J and K, show diffuse light pigmentation, masses of heavy pigmentation, and no nuclei. Two, L and M, show complete absence of nuclei.

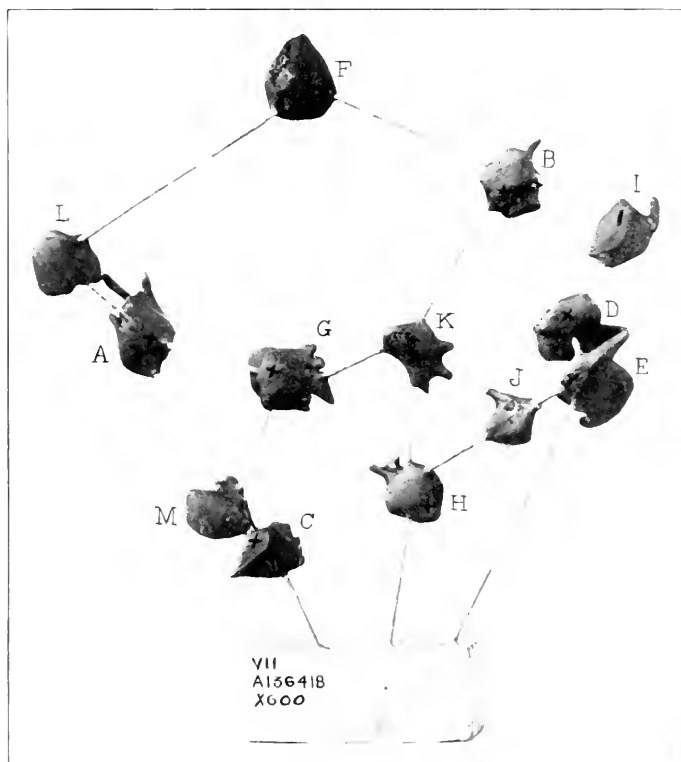


Fig. 8.

*Case VIII.*—A-135172, Fig. 9. (Case VII, Ref. 4.) This reconstruction is from a group of ganglion cells in the right superior cervical sympathetic ganglion of a female 39 years of age who had had moderate symptoms of exophthalmic goiter for twelve years with gradual abatement of symptoms for four years. Microscopically the total number of ganglion cells was apparently much reduced. The condition of those remaining is well represented in the reconstruction. None of the cells are normal. Nine of the ten are heavily pigmented. In the tenth, J, the nucleus is degenerated. Of the nine pigmented cells, five, A, B, C, D, and E, show quite normal nuclei. One, F, shows a partially degenerated nucleus. In one, G, the nucleus shows advanced degeneration, and two, H and I, are without nuclei.

*Case IX.*—A-53650, Fig. 10. (Case XV. Ref. 3.) This reconstruction is from a group of ganglion cells in the left superior cervical sympathetic ganglion of a female 21 years of age who after a year of suffering from severe symptoms of exophthalmic goiter had had double ligations of the superior thyroid vessels followed four months later by removal of the right lobe of the thyroid. Three years and eight months after the removal of her right thyroid, her left superior cervical sympathetic ganglion was removed. Examination of sections of this ganglion showed marked diminution in the number of ganglion cells. Of those which remained, few were normal. In the group of twelve shown, one only, A, is apparently normal. One, B, is normal except

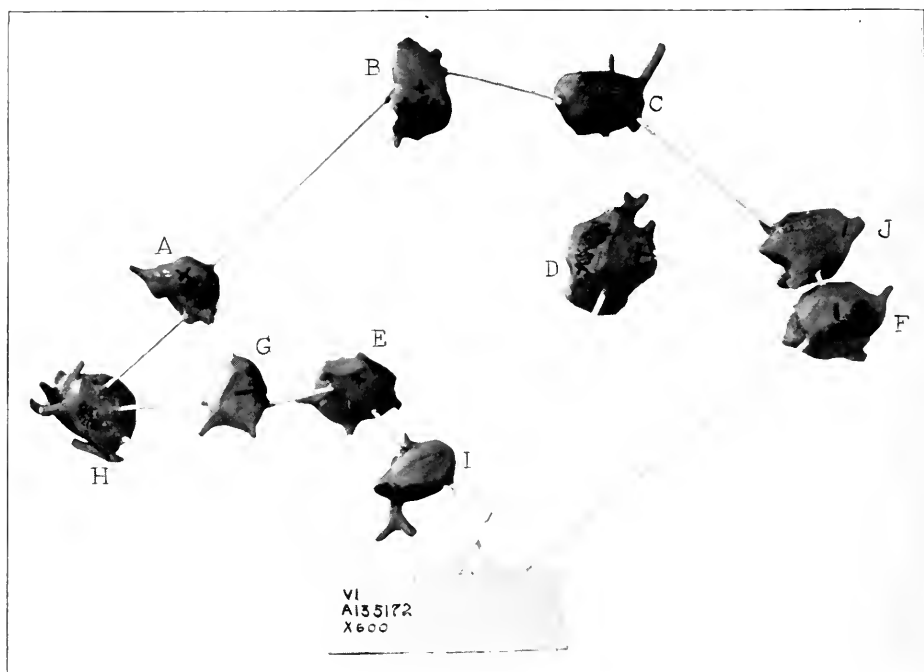


Fig. 9.

for a diffuse light pigmentation. One, C, shows intense local pigmentation but with an apparently normal nucleus. Three, D, E, and F, show similar pigmentation but with degenerated nuclei. Three, G, H, and I, show pigmentation with complete destruction of the nucleus. Two, J and K, are markedly atrophied, without nuclei and without pigmentation.

#### DISCUSSION.

These reconstructions of groups of ganglion cells have confirmed the impressions received from the microscopic study of series of sections of ganglia and made it possible to demonstrate their truth beyond a doubt. The material studied is all from patients so young as to preclude the possibility of senility as a factor in the changes observed in the ganglion cells. The material was all

in excellent condition when preserved. The methods of preservation, preparation, and reconstruction eliminate technical artefacts.

A study of the distribution of the cell changes in the groups illustrated shows that in every group there are cells which are apparently capable, of function, though in some instances this may be somewhat impaired in all the cells. At the same time in each group there are also cells which are apparently totally incapable of function. These two forms are in close relationship to each other. If we conceive of a distant muscle fiber or group of thyroid epithelial cells as controlled by a neuron, of which the ganglion cells of the functioning type are a part, then we must conceive of such muscle fiber or glandular epithelium as capable of being stimulated and, unless otherwise prevented, of functioning. Conversely, muscle fibers and glandular epithelial cells receiving

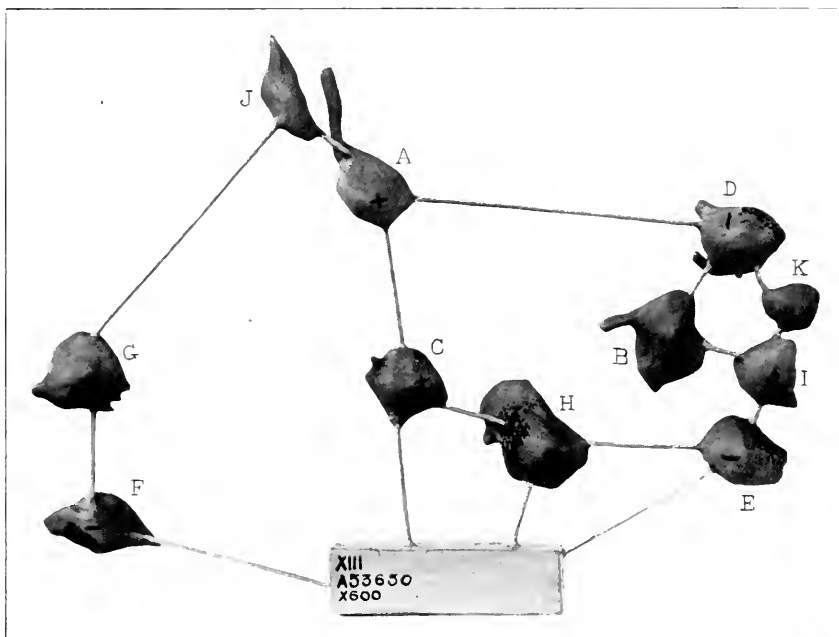


Fig. 10.

their nerve supply from neurons of which destroyed ganglion cells are a part, cannot receive their stimuli to function through such neurons. Unless another neuron vicariously assumes the function of the one in which the broken link exists, the muscle fiber or glandular epithelium must perforce go out of commission. The fact that functioning ganglion cells are in immediate proximity to nonfunctioning cells is in close harmony with the fact that in exophthalmic goiter we have muscle fibers and thyroid glandular epithelial cells in advanced stages of degeneration in immediate proximity to others of their kind which are apparently normal. That the changes in the ganglion cells may be secondary to primary changes in the muscle and glandular epithelium is rendered improbable by the fact that similar changes are not found in cervical ganglia of patients from whom a lobe of the thyroid has been totally removed.

for many months. Atrophic changes in the ganglion cells from ablation of the distant organ controlled by neurons of which the ganglion cells were a part are very different from the granular degeneration seen in cases of exophthalmic goiter. Nor do the changes seen in ganglion cells of persons of advanced age at all closely resemble those herein illustrated. Indeed there is every resemblance in the changes in the cervical ganglion cells in exophthalmic goiter to the changes in the ganglion cells in the spinal cord in anterior poliomyelitis and to the changes in the cortical cells of the cerebrum in meningitis. They may best be explained on the hypothesis of a specific primary infection of the ganglion itself.

NOTE ON THE TECHNIC USED IN THE RECONSTRUCTION OF GANGLION CELLS  
AT HIGH MAGNIFICATIONS.

The reconstructions were made from serial sections of tissue, stained by Ramon y Cajal's 1910 silver impregnation method and cut at 5 and 10 micra. In all, eighteen\* reconstructions of ganglion cells were made. Sixteen models were groups of ganglion cells reconstructed at a magnification of 600 diameters. Five of these were from human tissue removed at operation, eight from human tissue removed at autopsy, two were groups of cells from a goat, and one was of cells from a brook trout. Two models were reconstructions of single human ganglion cells at a magnification of 3,000 diameters. One of these showed a moderately degenerated cell, and the other a practically normal cell with its intracapsular processes and axone.

In making drawings for the above reconstructions at high magnifications, variations from the ordinary technic were found necessary. The principal difficulty encountered was that of tracing a single cell in serial sections. The usual method of selecting some definite landmark, such as a blood vessel, nerve process or some particular part of the ganglionic capsule which presented a comparatively uniform appearance throughout the series studied, proved successful for magnifications as high as 600 diameters. With magnifications of 3,000 diameters the cell studied covered almost the entire microscopic field, and no identification marks appearing throughout the series could be found. In order then to accurately trace a cell from section to section at this magnification, the entire series was drawn at a low magnification where definite landmarks or guides could be followed, and it was then possible to identify the different sections of a cell at much higher magnifications. When a section of a cell was located at a low magnification, it was exactly centered in the microscopic field, the higher power ocular and objective put in place, and the cell drawn.

The diameters of the normal, or nearly normal, cells were found to vary from 30 to 50 micra. Thus, in 10 micra sections, there were four or five sections to a cell, while in the 5 micra the average was from eight to ten sections. Cells showing advanced stages of degeneration were sometimes as small as 5 to 8 micra in diameter.

\*Only 10 are herein shown and described. The others will be published later in relation to other studies.

The alignment and transfer of the drawings to wax plates were done with the aid of translucent paper as described by Kashtshenko<sup>5</sup> so that a single section of a group of cells could be placed in exact registry over the section just proximal or distal to it. On the first section of a group two lines were drawn perpendicular to each other. Several crosses were placed in rather arbitrary relation to these lines. The second section then was fitted accurately over the first section, and these lines and crosses duplicated on it. The same procedure was followed throughout the series. Thus, though there might be a variation in the position of a section of a cell in a series due to an oblique cut, or perhaps to a natural variation, it was still accurately shown by these lines and crosses.

In transferring the drawings to the wax plates, the lines and crosses were also transferred. When the wax was cut, the marks were preserved in the form of bridges and guides and, when piled serially in the usual method, wire was substituted for the wax bridges.

In coloring the models the colors used were selected to conform as nearly as possible with the color which each cell stained by the silver impregnation method. Thus, in a single group, several shades were frequently used. The relative extent and depth of staining of the pigment is indicated by dots on the surface of the models. Each cell, if marked at all, bears a +, 1, or - sign, indicating

+ = Normal nucleus.

1 = Earlier stages of degeneration of the nucleus.

- = Advanced stages of degeneration of the nucleus.

No mark = No nucleus found in any of serial sections of the cell.

The wax plates were made for us in the Anatomical Department of the University of Minnesota through the kindness of Prof. Thos. G. Lee.

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# PIPERAZIN AND OTHER ORGANIC URATE SOLVENTS\*

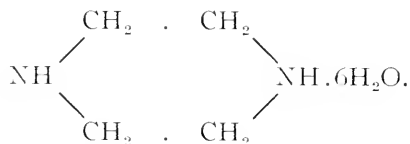
BY PAUL J. HANZLIK, M.D., CLEVELAND, OHIO.

IN a previous paper on hexamethylenamine (Hanzlik, 1916) a review of the literature revealed the practically negligible position of this drug among the so-called urate solvents, particularly when contrasted with such well known and tried alkalis as the bicarbonate and citrate of sodium. Special urate solvent properties have been ascribed to other agents. Among the best known and widely exploited, of these is piperazin. However, our knowledge of the behavior of uric acid and urates in the body and its fluids makes it difficult to understand why any special virtues should be ascribed to this product.

It is the object of this paper to examine the literature on piperazin and other urate solvents and to ascertain what, if any, scientific data exist to support the claims that are made. A proper appreciation of the chemical properties and the behavior of uric acid and the urates in the body is necessary in order to judge the data. For this the previous paper may be consulted. The complexity of the chemical behavior of the urates is further illustrated in recent contributions by Lambling and Debaussy (1914) and Haskins (1916). These emphasize the paradoxical behavior of the solvent power of urine itself for uric acid, and other properties, rendering positive deductions extremely difficult. It may be further stated that positive and optimistic statements regarding the therapeutic efficiency of the class of substances called urate solvents should be generally regarded as premature and unfounded, particularly if it is remembered that the clinical conditions for which these are frequently recommended are still more obscure. Bearing these uncertainties in mind, we may now proceed to examine the possibilities offered by piperazin as a urate solvent.

## 1. ADDITION OF PIPERAZIN TO PURE URATE.

Piperazin is a synthetic organic base obtained by the condensation of two ethylene with two amino groups. It forms soluble salts with uric and other acids. Its structural formula is



*Solubility of Piperazin Urate.*—Piperazin dissolves uric acid even in the cold, 1 part of the salt, piperazin urate, being soluble in 50 parts of water at 17° (Mayer and Schmidt, 1890). According to Mathews (1915) the piperazin salt of uric acid is much more soluble than the alkaline salts, the solubility

\*From the Pharmacological Laboratory, Western Reserve University, Cleveland. This review was prepared at the request of the Therapeutic Research Committee of the Council of Pharmacy and Chemistry of the American Medical Association.



being about 1 in 50 parts of water at 17°. Biesenthal and Schmidt (1891) give the solubility of various urates in water as follows: Piperazin urate 1:50; acid sodium urate 1:1200 at 15°; lithium urate 1:367 at 20°. Vicario (1902) reports that 1 part of piperazin urate is soluble in 45 parts of water at 18°, in 44 parts at 37°; 1 part of acid sodium urate in 1,136 at 18°, and in 581 parts at 37°. This is less than that reported by Vicario for sodium urate which was found to be 35 parts at 37°; somewhat higher, 59 parts, at 18°. 1 part of potassium acid urate was soluble in 666 at 18°, and 345 at 37°. Helbing and Passmore (1894) claim that piperazin can form an acid salt with uric acid, but that this never occurs in therapeutic practice. They claim further that neutral piperazin urate is the normal urate produced by the solvent action of piperazin on uric acid. It is said to be extremely soluble, 1 part piperazin urate dissolving in 50 parts water at 17°, in 40 parts at 38°. These data agree favorably with those reported by others as pointed out above. In attempting to compare the solubility of uric acid in piperazin in low or therapeutic concentrations, Helbing and Passmore worked with percentages that are altogether too high. They state that the concentration of 0.025% would approximately represent the percentage of piperazin in the blood after a dose of 15 grains (about 1 gm.). However, it is not correct to assume the distribution of the drug to be limited to the blood only. The tissues as a whole should be considered. The weight of the individual is not stated, but assuming it to be an adult of about 70 kilos, the concentration in the tissues, as a matter of fact, would be about 0.001% and 0.002% for a 50 kilo individual.

*Comparison With Lithium and Effect of Neutral Salts.*—In test-tube experiments, according to Cushny (1910) and Schmiedeberg (1909), the solubility of uric acid in piperazin is greater than that of lithium and borax. Heinz (1907) states this to be about twelve times that of lithium carbonate; Helbing and Passmore (1894) about seven times. This is denied by Van der Klip (1892) who found the solvent power of piperazin (concentration unknown) to be less than that of lithium carbonate between 16° and 36°. Plugge (1895) states that it is only in high concentrations that piperazin is a better uric acid solvent than lithium. In dilute solutions it is less so. Ortowski (1900) found that piperazin in aqueous solution at 37.5° possessed less solvent power for uric acid than lysidine but acted somewhat more favorably than sodium bicarbonate.

In the presence of even small quantities of sodium chloride (less than 1%) this solubility is lessened, hence in urine the solvent power of piperazin is almost completely lost. Morhorst (1896) states that the addition of sodium chloride, sodium sulfate and other neutral salts to a piperazin solution of uric acid causes precipitation of urate from the piperazin urate. According to Penzoldt (1900) the solvent power of aqueous solutions of piperazin is lessened by the addition of urine. He found that a uric acid stone placed in a 1% solution of piperazin and incubated for 24 hours was dissolved along the edges only. Nicolaier (1899) thought that piperazin dissolved uric acid and urates less readily than hexamethylenamine. On the other hand, Stevens and May (1911) found that piperazin was a better solvent than hexamethylenamine.

It may be concluded that piperazin forms a salt with uric acid called piperazin urate, whose solubility is about 1 in 50 parts of water, and that this is diminished by the addition of urine and weak concentrations of neutral salts. Lithium apparently is a better solvent for uric acid *in vitro* than piperazin in low concentrations.

## 2. ADDITION OF PIPERAZIN TO URATE CALCULI.

Casper (1897) observed that as solvents for calculi piperazin, lysidine and hexamethylenamine were no more effective than urine or water alone, but glycerin was superior. Biesenthal and Schmidt (1891) observed that 1% piperazin dissolved a urate stone better than lithium carbonate, sodium carbonate, and borax of the same strength. Gordon (1894) treated natural vesical calculi with piperazin, borax, sodium bicarbonate, lithium citrate and potassium citrate. One per cent piperazin was found to possess considerable solvent power if the contact was long enough. The calculi were softened, rendered mushy and it was conceived that in the body they would be somewhat more easily "absorbed." When 50 mgm. of a powdered stone consisting of 59.19% uric acid was placed into 1% piperazin for 24 hours at 39°, 48 mgm. were dissolved while the other solvents under the same conditions dissolved only 25 to 35 mgm. This led to the conclusion that the solvent power of piperazin under like conditions is greater than that of borax, lithium citrate, sodium bicarbonate or potassium citrate. On the other hand, Van der Klip (1892) found that uric acid calculi are not dissolved by piperazin as readily as by lithium carbonate.

Ebstein and Sprague (1891) used piperazin as a solvent in the course of analytic procedure and found that gouty tophi consisting of urate can be dissolved by 1:1000 piperazin *in vitro*. Finzelberg (cit. Helbing and Passmore, 1894) is said to have made experiments demonstrating the solvent power of piperazin for different kinds of stone, and from this he suggested the use of the drug in the treatment of arteriosclerosis, "compound" sclerosis and atheromatous changes of the aorta.

It may be concluded that piperazin, in rather high concentration, possesses some power to dissolve calculi, but the extent of this may be regarded in some cases limited and doubtful, due in part, no doubt, to the uncertain and variable composition of calculi.

## 3. SOLUBILITY OF URIC ACID AND URATE IN SERUM CONTAINING PIPERAZIN.

The existing scientific data, though limited, indicate this to be of no practical importance.

Sir W. Roberts (Croonian Lectures, 1892) stated that the addition of 0.1 to 0.2% piperazin to blood serum or synovial fluid had no effect in increasing the solvent power of these media on sodium biurate, nor did it retard the precipitation of uric acid from serum and synovial fluid.

Bohland (1894) found that 50 c.c. of serum (horse and calf) containing 1% piperazin dissolved about 0.1 gm. uric acid at ordinary room temperature, that is, about 0.2%. From this solution (on standing), or when serum is added

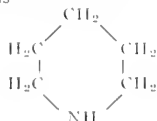
to an aqueous solution of piperazin, a flocculent precipitate is formed consisting chiefly of biurate. The solubility of potassium acid urate in 1% piperazin was found to be the same as for uric acid. Tunicliffe and Rosenheim (1898) found the solubility of sodium biurate in beef serum to be 1:31,000; other solvents in order of increasing efficiency being lysidine 1:25,000; hexamethylenamine 1:14,000; and piperidin\* 1:12,000. The solubility in serum alone was found to be 1:60,000, which, if given the value of 1, would give piperazin a value of 1.9. The true reaction of the serum in all these experiments is not indicated, and as it might conceivably become strongly alkaline (by decomposition) the value of the results is considerably minimized.

#### 4. SOLVENT ACTION OF PIPERAZIN URINE ON ADDED URIC ACID.

The solubility of uric acid in piperazin urine is much less than in aqueous solutions and it is markedly influenced by dilution and the reaction. Unfortunately the effects of dilution and reaction have been overlooked by a majority of the earlier observers.

Helbing and Passmore (1894) found that urine interferes with the urate solvent action of piperazin. They observed that 100 c.c. of 1% piperazin urine dissolved only from 0.0065 to 0.03 gm. of uric acid in 72 hours; 100 c.c. of 0.1% piperazin urine dissolved from 0.0004 to 0.0017 gm. uric acid in 72 hours. Goodbody (1896) observed that the addition of 0.1 gm. piperazin to 220 c.c. urine, 0.2 gm. in another, which regularly deposited urate, dissolved the urate to the extent of 0.01%. The average uric acid content of 5 urines before the addition of piperazin was 0.058%, and this was increased to 0.067% by the addition of 0.2 gm. of the drug. The addition of piperazin (0.085 to 0.68%) to urine (100 c.c.) containing added uric acid caused variable quantities (28.12 to 233.32 mgm. respectively) of the latter to go into solution (Haskins, 1916). This was also true of dilute and less dilute urines after the administration of the drug and whether the reaction was truly acid or alkaline. Dilute urines without the drug but possessing the same reactions frequently dissolved more uric acid than when they contained piperazin. Generally, alkaline urines dissolved more uric acid, but it is also true that Haskins found considerable uric acid dissolved in a few acid urines. After the administration of 1 to 2.5 gm. daily to 2 individuals during the course of 3 days, Heubach (1891-92) observed that the piperazin urine would not dissolve any added uric acid even at higher temperatures and with prolonged action. Meisels claims that 70 per cent of added uric acid (presumably to urine) was dissolved at room temperature. However, complete data are not cited in the report which was available. Mendelsohn (1892) could demonstrate no solvent action on calculi or uric acid in piperazin urines and concluded from this that no better results

\*Piperidin is a rather active substance pharmacologically, causing a rise of blood pressure in cold blooded animals, cardiac inhibition with large doses, and a curare-like action on motor nerve endings. According to Oesterle (1909) the formula is



could be hoped for from injections into the bladder, because such solutions can be retained for 2 to 3 hours only, and the solvent effect would decrease with the advent of urine, rendering the solvent action of piperazin practically useless. Penzoldt (1900) states the uric acid solvent properties of aqueous solutions of piperazin are lessened by the addition of urine. Tuncliffe and Rosenheim (1898) found that 100 c.c. of a 0.2% piperazin urine dissolved 0.058 gm. or 11.6% of 0.5 gm. of added uric acid; 100 c.c. of 0.1% piperazin urine dissolved 0.0415 gm. or about 8.3%. Urine alone dissolved extra uric acid, and if the value of 1 is assigned to urine, the value for 0.2% piperazin would be about 13.5, for 0.1% it would be 5.7. Piperidin and lysidine were found to be more effective, hexamethylenamine less efficient. Weiss (1900) states that urates of piperazin and lysidine are soluble in water, but not in urine, an objection that cannot be held against urosin (to be considered later).

#### 5. EFFECT ON URATE DEPOSITS.

*Experimental Deposits in Birds.*—Deposits in the various organs of birds produced by foreign substances have been regarded by some investigators as the clinical equivalent of urate deposits in various disease conditions in human individuals, and gouty tophi. It is apparent that the conclusions drawn from such uncertain analogies furnish a very insecure basis for the therapeutic efficiency of the so-called urate solvents in gout and similar conditions. Nevertheless, we may briefly allude to the effects of piperazin in this interesting avian condition.

Fawcett (1894) observed that deposits produced in pigeons by gradual injections of chromate disappeared after the injection of 0.001 to 0.0005 gm. piperazin. The kidneys were found to be swollen, pale or mottled, and Fawcett suggests that as a result of the piperazin injections the outlet for uric acid was blocked and the uric acid "deposited" in the tissues. Presumably retention of uric acid rather than actual deposits was intended. Practically the same was observed by Meisels (1893), who found that not only could piperazin prevent the formation of deposits but also remove those already formed. Whereas control animals showed a parenchymatous nephritis and uric acid concretions in the tubules, those treated with piperazin were free from these. Lithium carbonate and sodium bicarbonate were found to be without any influence on urate deposits in birds. Waucomont (1912) produced experimental gout in chickens by the feeding of horse flesh, and observed the effects of various urate solvents on this. It was found that piperazin, lycetol, sidonal and potassium iodide produced no influence on the excretion of uric acid, and most of these agents also did not influence the course of the clinical condition. The disappearance of joint swellings, deposits and symptoms of gout in an eight year old parrot following the administration of piperazin (0.08 gm. daily) is reported by Zimmermann (1901). However, since the patient received bicarbonate internally, and salicylated collodion locally together with the piperazin treatment, it cannot be concluded which, if any, of the beneficial effects were due to piperazin alone. Biesenthal (1893) claims to have confirmed the work of Meisels (1893), and thereby procured support for the therapeutic claims made

for the drug in "uric acid diathesis." Both chickens and pigeons were used. About 7% of the animals injected with chromate failed to respond with demonstrable urate deposits. Eighty per cent (16) of the chickens and 82% (14) of the pigeons treated with piperazin showed no deposits. However, since a considerable number of animals fail to give evidence of deposits with repeated injections of chromate, a large element of uncertainty must exist in this form of experimentation, likewise in the conclusions drawn therefrom. Proportionately for human individuals, the doses used by Biesenthal were very large, a total dosage of 27 gms. having been administered. Lithium carbonate, sodium phosphate and borax are reported by Biesenthal not to have influenced the deposits.

*Deposits in Gout and Other Conditions.*—According to Meyer and Gottlieb (1914) the view that piperazin and lysidine cause removal of retained uric acid in the tissues has been found to be erroneous. Mordhorst (1892) observed that piperazin would be a good solvent for urates, but a 1% solution, which is effective, is inconceivable in the body, the concentration in blood being about 0.0125%. A disappearance of uric acid crystals in standing urine was noticeable, but the same followed more completely with the use of mineral waters. Mordhorst points out that the excretion of piperazin after internal administration, its destruction in part, and the doubtful affinity displayed by piperazin for uric acid being greater than that of the alkaline salts of the blood are important additional reasons why such agents as piperazin and lysidine cannot act favorably on urate deposits in the living organism. In a later communication, Mordhorst (1896) emphasizes the absurdity of expecting the alleged solvent effects of piperazin. He states that piperazin cannot act in the living organism because of the homeopathic concentration of the drug in the body, about 1:54,000, and it must be remembered that a  $\frac{1}{2}$ % solution dissolves uric acid very little,  $\frac{1}{4}$ % still less and  $\frac{1}{8}$ % not at all. "It is therefore not understandable how anyone can earnestly believe that a solution of this salt, 1:54,000, can have even the slightest uric acid solvent action." Yeo (1909) quotes Sir Roberts to the effect that both piperazin and lithium cannot prevent the formation of uratic deposits.

#### 6. EFFECT OF THE ADMINISTRATION OF PIPERAZIN ON URIC ACID AND URATES.

According to Haskins (1916), who has furnished the best scientific evidence on this subject, piperazin in small doses does not affect the excretion of uric acid in urine. Using proper controls and carefully observing the reaction of the urine (hydrogen-ion concentration), Haskins found that when piperazin is administered in very large doses only (extra therapeutic) there is some increase in the excretion of uric acid. However, this was more effectively produced by the administration of ordinary alkalies such as citrate and bicarbonate of sodium. The use of large doses of piperazin according to some clinical reports (to be referred to later more fully), is perhaps harmful, and, therefore, not justified, particularly in view of the fact that the same object may be attained by the use of well tried and relatively harmless alkalies. The work of

Haskins is confirmatory of certain of the older observers, although it must be admitted that there is an element of uncertainty in their work owing to the lack of proper dietary and other controls, the failure to realize the importance of the reaction of urine, the influence of diuresis and the variable solvent power of urine itself. An exception to the older work is that of D. D. Stewart, and it agrees essentially with that of Haskins.

D. D. Stewart (1893) showed quite conclusively that medium-sized doses of piperazin used over long periods are without effect on uric acid excretion. This observer carefully studied the volume of urine, specific gravity, acidity, urea and uric acid excretion daily before and after the administration of the drug for several days in 3 cases of renal stone, using also proper fluid intake and, apparently, dietary controls. The dosage of piperazin was about 24 grains (1.5 gm.) daily for 14 days in each case. Under these conditions there was no increase in uric acid elimination demonstrable and attributable to the drug, also no increase in urea excretion. Later observations by Stewart (1894) with even larger doses of the drug, 70 grains (nearly 5 gm.), daily, confirmed his previous results. That is, the excretion of uric acid, urea and chloride remained uninfluenced.

The following abstracts are cited against piperazin as a urate solvent: According to S. Fraenkel (1912) objective experiments indicate that the uric acid solvents, such as piperazin and others pertaining to its class, are worthless and when effects are seen, these are produced more by other conditions. Percy May (1911) is of a similar opinion; namely, that while some benefit may be derived from the administration of the piperazin class of urate remedies, this is not due to their solvent action on the uric acid. The employment of these bases as uric acid solvents is fallacious because there is always sufficient sodium present in the body to form the sparingly soluble sodium urate. Further, owing to the detrimental effects of small quantities of sodium chloride on the solvent power of piperazin, and the fact that it is to a large extent destroyed in the body, leaving only a small quantity unchanged in the urine, the uric acid solvent action of the drug on internal administration seems very problematic (Heinz 1907). According to Cushny (1910) the urine of patients receiving piperazin has no more solvent action on uric acid than urine itself; and whatever piperazin escapes into the urine is in combination with the stronger acids and not uric acid. Hare (1905) states that repeated clinical observation has shown that the administration of the drug causes an increase in the amount of urea in the urine with decrease in uric acid indicating that under its influence oxidation is more complete. He gives no data. Various conditions in which piperazin is used and recommended are, in Hare's experience, not benefited by the administration of the drug. Likewise Penzoldt (1900) claims he could recognize no influence by piperazin medication on the decrease of uric acid excretion. Poullson (1912) doubts, (for well known reasons, previously mentioned), whether piperazin has ability to dissolve uric acid in the organism.

In a case of leukemia which showed increased uric acid excretion and urate deposits in urine, Bohland (1894) found that during piperazin treatment, as well as with potassium bicarbonate, and when no urate solvent was administered,

the excretion remained the same. In spite of large doses, piperazin did not affect the deposits but these were removed by potassium bicarbonate. Ebstein and Sprague (1891) administered 14 grams of piperazin within 7 consecutive days and carefully observed the quantity of uric acid excreted together with proper control of dietary and fluid intake. There was only a slight, and practically negligible increase in uric acid excretion, the urine output during this time being variable. Fauvel (1908) found that small doses of piperazin (1 gm. daily) diminished the excretion of endogenous uric acid, the diet during this time being purin free, but when the dosage was increased (2 to 4 gm. daily) the excretion of uric acid was increased, but not above the quantity before piperazin was ingested.

Ortner (1908) claims that piperazin seems in some cases to be effective as a therapeutic agent, its action being explained by its power to dissolve uric acid. However, Ortner emphasizes that he never prescribes piperazin alone, but with a bottle of Seltzer, Preblau or soda water, to which, no doubt, may be ascribed a considerable, if not the greater, share of the supposed solvent action. Using the colorimetric method of Folin, Robertson (1914) observed that piperazin as well as a number of other substances (pituitary, saline,  $\text{Ba Cl}_2$ , caffeine and  $\text{Na}_2 \text{S O}_4$ ) produced in hens no changes in uric acid excretion sufficiently independent of urine flow to suggest any specific effect of the agents used.

The following is a summary of reports favorable to piperazin, though scientifically not acceptable:

Abl (1913) claims that piperazin produced an increase in the urinary excretion of uric acid, and the same is reported by Abl for a number of diverse agents such as mustard, arsenic, colchicine, thorium X, sulfur, choline, chloral hydrate, neurine and strontium! The mechanism of action, Abl believes, is located in the intestine. However, it seems quite probable that diuresis at least in part (contrary to the claim of the author) and other factors, such as disease, destruction, circulatory changes, etc., were responsible for the results reported by Abl. Attaix (1896) made the claim that the quantity of uric acid and urates is augmented proportionately to the urea. This and other claims of this author are not supported by any discoverable scientific data. The effect of piperazidin on the excretion of uric acid was studied by Bardet (1891), but with no urinary or dietary controls, rendering it impossible to draw any conclusions from the work. Goodbody (1896) administered piperazin in 1 gm. daily doses for 9 days, and 2 gm. daily during the next 5 days, maintaining the diet and fluid intake constant. Under these conditions uric acid excretion increased from 0.31 to 0.34 gm. on the 1 gm. dosage, and eventually increased to 0.37 gm. with the 2 gm. dosage. Goodbody concluded that administration of piperazin increases the elimination of uric acid, not by increased formation, but by rendering the blood more capable of removing it from the tissues by increasing its solvent power. However, it must be urged that these differences are small and within ordinary variations, as indicated by Goodbody's own results before the administration of the drug. Finally, granted that piperazin

produced this increase in the excretion of uric acid, it is so small and the dosage so large, and the duration of administration so long that it would be practically valueless, uneconomical and undesirable.

## 7. EFFECT ON DIURESIS.

There are many statements in the literature to the effect that piperazin can favorably influence diuresis, but the scientific evidences for this are deficient. Attaix (1896) makes the statement that piperazin lessens the density of urine and acts as a powerful diuretic. However, in view of the fact that only imperfect data are cited in connection with a single observation of his own, Attaix's conclusion is unjustified. The more careful observations of Bohland (1894) showed that piperazin did not change the quantity of urine voided in 24 hours. Ebstein and Sprague (1891) also found that while the urine output during administration of piperazin was variable, there was no marked change from diuresis before piperazin. Fraenkel (1912) referring to the general group of urate solvents, including piperazin, states that these agents possess chiefly a diuretic action, and dilution of urine facilitates excretion of uric acid. This statement, however, is unsupported by any data. Goodbody (1896) administered piperazin (1 to 2 gm. daily) for several days (14) to an individual whose urine exhibited deposits of uric acid gravel regularly (using proper dietary and fluid intake controls) and found that 1 gm. of piperazin increased the volume of urine from 1103 c.c. to 1476 c.c., and 2 gm. caused a further increase to 1680 c.c. A closer inspection of Goodbody's tables reveals the fact that diuresis before administration of the drug was variable, and on certain days before treatment with piperazin the values are as great as those during piperazin periods. It is hardly permissible, therefore, to ascribe any great importance to these results, particularly since the loss of water by other channels (sweat and the intestine) have not been considered. Heubach (1891-92) administered 2.5, 1 and 2 gm. piperazin on the first, second, and third days, respectively, to two different individuals and observed that only a slight increase in urine output occurred with no diminution in specific gravity. Kobert (1897) states that all of the following are diuretic in doses of 1 to 2 gm. per day; piperazin, lycetol, lysidine and hexamethylenamine, and that they may act in part by removal of water; but the evidence for this is lacking. D. D. Stewart, (1893) administered 18 to 30 grains (1.1 to 2 gm.) daily of piperazin over long periods of time (14 days) consecutively in 3 cases of renal stone and observed an increase in urine volume in one case which could be explained by a hydronephrosis present that disappeared following an operation for removal of the stone. In a later communication, D. D. Stewart (1894) claims that two different preparations of piperazin used had invariably a diuretic influence. The elimination of urea and chloride at the same time remained unaltered. Wittzack (1893) also claims that piperazin (using about 10 gm. per day) caused an increase in diuresis. However, it is not clear whether the fluid intake was controlled. Umpfenbach (1891) is reported to have observed a favorable influence on the quantity of urine excreted, and on the nerves and muscle of the bladder.



## 8. EFFECT ON THE REACTION OF URINE.

The scientific data on this are conflicting, although most clinicians seem to be of the opinion that the reaction is unchanged. Aside from the observations of Haskins (1916) there are no data in the literature, so far as I know, regarding the true reaction (i.e., hydrogen-ion concentration) of urine as influenced by piperazin. Haskins found that the direct addition of piperazin or lysidine to acid urines lowers the hydrogen-ion concentration (from 5 to 7.4 with a piperazin concentration of 0.68%), i.e., the reaction was alkaline. However, after administration, the reaction of urine was uninfluenced and frequently remained acid. The reactions referred to in the following papers are presumably in many cases intended to convey changes with litmus, in others, by titration. Owing to these uncertainties, the value of the abstracts is considerably diminished. Nevertheless, it seemed worth while to refer to them.

Biesenthal (1892), citing the advantages of piperazin over ordinary alkalies and waters, states that after prolonged administration of piperazin the urine remains acid, but he gives no scientific data. Bohland (1894) claims that the administration of piperazin in a case of leukemia slightly lowered the acidity of urine. Goodbody (1896) made observations as follows: The addition of 0.1 gm. piperazin to 220 c.c. of urine from a patient suffering with uric acid gravel, and 0.2 gm. to the same volume of urine from another patient whose urine regularly deposited urate, increased the acidity from 0.41% to 0.44% (NaOH) in 24 hours and 0.39% to 0.40% in 36 hours, respectively. In 5 cases the acidity of the urine was reduced from an average of 0.39% to 0.38% by the administration of 0.2 gm. piperazin. These differences are so small as to be practically negligible. Further observations by Goodbody on the effect of prolonged administration of piperazin in doses ranging from 1 to 2 gm. per day gave the following results: The average during normal days was 0.46% NaOH, and this fell to 0.36% on 1 gm. piperazin daily; 0.2 gm. piperazin daily caused a further fall to 0.30%. The conclusion reached was that piperazin has the power to diminish the acidity of urine. It must be pointed out, however, that this does not necessarily mean there was a reduction in the true reaction of the urine. Fauvel (1908) found that the reaction of urine from individuals on a purine free diet was unchanged by small (1 gm.) or larger (2 to 4 gm.) daily dose of piperazin. The administration of 2.5, 1 and 2 gm. of the drug on the first, second, and third days, respectively, by Heubach (1891-92) had no influence on the reaction of the urine, which remained acid. In the experience of Mordhorst (1892) piperazin causes only a small reduction in urinary acidity. In his observations, D. D. Stewart (1893, 1894) found that the acidity of urine was not appreciably affected by the administration of piperazin. On the other hand, Wittzack (1893) claims the acidity of urine is lowered, the urine, however, never becoming neutral or alkaline. It is not clear whether the influence of dietary in Wittzack's work was excluded or not.

## 9. EFFECT OF THE ADMINISTRATION OF PIPERAZIN ON CALCULI.

This feature of piperazin therapy has undoubtedly been exaggerated. The statements and opinions held regarding the favorable influence that piperazin treatment is alleged to possess on the removal and solution of calculi are unsupported by a single iota of scientific evidence. For instance, the claim of Biesenthal (1892) for the superiority of piperazin over alkaline mineral waters as a preventive of stone formation is made without foundation of fact. He also makes the surprising claim that alkaline mineral waters lead to stone formation because of phosphate precipitation. Cushny (1910) observes that piperazin has not been shown to be of any value in the treatment of calculus. Referring to its use as a preventive of the formation of renal and vesical calculi and other conditions, Hare (1905) states that he has failed to obtain any results from the use of this drug in practice. Fawcett (1894) made observations as follows: An individual was given 15 grains (about 1 gm.) piperazin daily for 2 weeks. The 24-hour specimens of urine (preserved with choloform) were collected and allowed to drip over a stone for the next 24 hours kept at body temperature. Two such experiments are reported. In one experiment the stone weighed 2.4 gm. before the experiment and 2.404 gm. at the end of the first week during treatment. The same stone in the second experiment weighed 2.4435 gm. before and 2.6155 gm. at the end of the second week. The stone contained 78.5% uric acid by analysis. The same individual's urine was again collected for 14 days, piperazin (1:1000) added to it and a portion of the same stone used in the previous experiments was added to it and another portion to water containing 1:1000 piperazin. No solvent action was observed by piperazinized urine, but complete solution took place in the water. Fawcett's simple though ingenious experiments demonstrate clearly the inefficiency of piperazinized urines, obtained either by administration, or by addition of the drug directly, to dissolve urate stone.

Maramaldi (1897) reports rather optimistically that piperazin has the power to give specific symptomatic relief in renal colic due to calculus, because, as he puts it, "the patient claimed to feel like a new born man" after 1 gm. piperazin per day. Morphine and other measures had been previously used, however, without effect. No objective evidence is offered. From experiments on pigeons, in whom it is claimed experimental urate deposits were successfully prevented and removed by the administration of piperazin, Meisels (1893) declares piperazin can easily dissolve uric acid calculi in dilute solutions, being superior in this respect to sodium borate and phosphate, and that the results with the drug clinically are gratifying. No clinical data are furnished by Meisels. Beneficial symptomatic relief in individuals suffering with calculi is claimed by D. D. Stewart (1894) whose work with the urate solvents is more critical than that of his contemporaries. However, Stewart makes it plain that the beneficial action cannot be explained by uric acid excretion and prefers to leave the mechanism of action unexplained.

The observations of Mendelsohn (1892) cast doubt on the alleged solvent action of piperazin for calculi. Mendelsohn found that while piperazin in water

dissolves uric acid calculi, no solution either of stones or uric acid takes place in piperazinized urine. He explains also that no better results can be hoped for from injections of solutions of piperazin into the bladder because they can be retained for 2 to 3 hours only and the solvent effect would decrease with the advent of urine, rendering the solvent power of piperazin practically useless, all of which clearly emphasizes the fact that when piperazin is used under conditions of the body no beneficial effect, or solvent action of the drug, is demonstrable. Mordhorst (1896), whose work has been cited in another place, also clearly explained why no beneficial therapeutic action may be expected from the so-called urate solvents. Penzoldt (1900) concurs in this view, also Poulsson (1912). H. C. Wood (1902) is of the opinion that while piperazin may be of service as a solvent of uric acid gravel, it will not dissolve a ready formed calculus in the bladder.

#### 10. EFFECT OF PIPERAZIN IN GOUT.

The uncertain role that uric acid plays in the etiology and symptomatology of gout has not prevented certain clinicians from frequently recommending the so-called urate solvents as beneficial therapeutic agents in this condition. In view of the etiologic uncertainty, the use of such agents is certainly irrational. That the value of so-called urate solvents in gout may be considered negligible is amply confirmed by the reports in the literature which are conspicuous for lack of scientific evidence; judgment being further impaired because of the influences of other attending circumstances, various unknown factors, and allied medication. The following abstracts, therefore, possess only the value that may be attached to opinion unsupported by scientific evidence.

Heinz (1907) states that piperazin has been administered very much in gout and "uric acid diathesis" without promising results. Penzoldt (1900) agrees with this. Because, as Kobert (1897) supposes, the so-called urate solvents act as diuretics, it is suggested that they act in gout by removal of water. However, Kobert's supposition regarding diuresis is not borne out by the facts. D. D. Stewart (1894) thinks that piperazin gives relief in gouty attacks. He is not clear as to how this is brought about, but he agrees that it certainly cannot be explained by any influence on the excretion of uric acid. Zimmermann (1901) reports gout in a parrot as having been permanently benefited by the administration of piperazin. However, the bird also received sodium bicarbonate internally, and salicylated collodion locally, rendering it, therefore, rather difficult to ascribe the beneficial effects observed to any one particular agent. Ataix (1896) reports a case of gout, which had previously been treated with colchicum and salicylate, but without response. The attacks, it is claimed, were prevented by piperazin. Biesenthal and Schmidt (1892) cite 5 cases reported by Bardet who is said to have demonstrated an increase of uric acid excretion in gout, but neither the data nor the conditions under which these were obtained is mentioned.

In contrast to all this may be cited the paper of Fawcett (1894) as the only piece of scientific evidence available. Using the Hopkins quantitative method for uric acid, and suitable controls, Fawcett found that piperazin ex-

erted no effect on the excretion of this metabolite in gout, or on the relief of the symptoms. He concluded that piperazin is not as valuable as certain drugs already recognized for the treatment of this condition.

#### 11. UNDESIRABLE ACTIONS OF PIPERAZIN.

Several reports in the literature indicate that the administration of piperazin may be attended with or followed by albuminuria, urticaria and other undesirable objective and subjective symptoms. Albuminuria due to piperazin has been claimed by Rörig (1893). Rörig reported repeated albuminuria in an individual who had had nephritis, but did not show albuminuria before the administration of the drug. Slaughter (1896) reports symptoms of collapse in an individual following the administration of 20 grains in a pint of water. Biesenthal (1891; 1893), however, claims that large doses of 6 gm. cause no disturbance of renal function. In criticizing the report of Rörig, Biesenthal states that the picric acid reagent for albumin also gives a precipitate with piperazin which appears in urine after administration, and therefore is not characteristic. Bradford (1892) observed urticaria in a patient with gouty tendency following the ingestion of about 1 gm. of piperazin, but inasmuch as the individual complained of digestive disturbance and had also received phenacetin, salol and local applications it is apparent that no definite conclusions regarding piperazin alone can be made. Headache and some vomiting after doses of 2.5 gm. were experienced by Heubach (1891). In phloridzinized dogs piperazin completely inhibits the glycosuria, and after 14 days' treatment of a diabetic patient with 1 to 1.5 gm. doses (total 3), the sugar excretion fell from 8 to 3.3% (Hildebrandt, 1893). The activity of hydrolytic ferments was also found to be checked. Gruber (1893) reports similar results. Rabbits tolerate 0.5 to 1 gm. hypodermically according to Van der Klip (1892). D. D. Stewart (1894) states that following the administration of 70 grains of the drug per day there were intermittent, clonic spasms of extremities, muscular prostration, incoherence, incoordination, tremors, and these symptoms disappeared 30 hours after onset. Tremor, malaise and nausea were observed with smaller doses. According to Wittzack (1893) subcutaneous injections of piperazin are painful. Wood (1902) claims to have observed muscular weakness and general depression following continuous exhibition of large doses. Therapeutic doses in man, however, produced no symptoms. Van der Klip (1902) observed that sufficiently large doses of piperazin cause vomiting, irregular breathing, general muscular weakness and relaxation. The dissociation of oxygen from hemoglobin was lessened in a concentration of 1:5000; a 0.5% solution favored the coagulation of blood and the action of peptonizing ferments was checked. Bohland (1894) also claims piperazin lessens the oxygen liberating property of hemoglobin because the spectroscopic band of oxyhemoglobin remained unchanged for days and weeks. According to Bohland piperazin is also an antiseptic and causes hemolysis. On the other hand, Cushny (1910) states that piperazin seems to induce no symptoms in man or animals even when used in large quantities.

## 12. FATE AND EXCRETION OF PIPERAZIN.

This is of importance in connection with the therapeutic use of the drug as a urate solvent, for if piperazin is largely destroyed in its passage through the body, much could not be expected of its supposed solvent action in urine. According to Mordhorst (1898) excretion of the drug itself occurs in urine, but so much is destroyed that a considerable reduction of the concentration occurs in the body. Van der Klip (1892) detected the drug in 4 hours in rabbit's urine. Neubauer and Vogel (1898) state that the greater part of the drug is excreted in a few hours; the excretion lasts a few days, and the drug appears unchanged in the urine. According to Wood (1902) piperazin is rapidly absorbed and eliminated by the kidneys producing a reddish-brown urine. With the aid of the bismuth-iodide reagent, Helbing and Passmore (1894) claim to have determined that the greater part (11 grains) of 15 grains of piperazin was excreted unchanged. By means of the same reagent, Zimmermann (1901) demonstrated the presence of piperazin in the cloacal contents of a parrot. Biesenthal (1893) claims piperazin is excreted into the urine as such. Cushny (1910), however, states that but very little of the drug reappears in urine, and what escapes in this way is in combination with the stronger acids and not uric acid. Fraenkel (1906) states that piperazin passes unchanged in urine, and is excreted rather rapidly, oxidation also taking place. The opinion in general seems to be that piperazin is decomposed to a certain extent and bound with acids in the body, and in this way robbed of any activity it may possess (Poullsson, 1912; Heinz, 1907).

## 13. OTHER URATE SOLVENTS.

*Lycetol*.—Chemically this is dimethyl piperazin tartarate. Its structural formula is

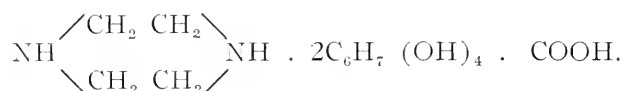


The behavior of lycetol in the body differs in no particular from piperazin (Fraenkel, 1906). Anthony (1895) claimed beneficial results in 6 cases of gravel. However, in the absence of any data whatsoever, this report may be regarded as a mere testimonial. Basile (1901) observed that lycetol can dissolve added uric acid, but that it is variable, the differences being due to variable qualities of urine. Added to urine in the concentration of 1%, lycetol dissolved about 0.7% uric acid, whereas after administration, the excreted portion in urine dissolved only about 0.4%. Lycetol, therefore, loses considerable uric acid solvent power by passing through the body. So far as urate calculi are concerned, 1% lycetol dissolved about 0.15 to 0.25% of uric acid, but had only a weak effect on mixed calculi. Basile also studied the effects of the administration of lycetol to 26 patients and concluded as follows: Diuresis was augmented (from 910 to 1100 c.c.); gravel disappeared from urine; the quan-

tity of uric acid excreted remained small or uninfluenced in the beginning, but later was augmented; clinically the patients who suffered with "uric acid diathesis" became improved; the specific gravity of urine was not appreciably reduced; urinary acidity was diminished, some urines becoming neutral, others alkaline. Beneficial results in gout and sciatica are also claimed by Basile. While the relief in the various conditions was attributed by Basile chiefly to the urate solvent power of lycetol, this can hardly be the case altogether because of the small and uncertain uric acid values reported by him, the uncertainty of factors influencing this, and because of the possibility of spontaneous relief in these conditions without drugs. In much the same way may be regarded the symptomatic relief in gout and sciatica reported by De Tollenaere (1897).

Hoven (1898) reported beneficial results with 1 gm. of the drug in gouty individuals, and attributed this favorable action to increased alkalinity of the blood and urine, but these conclusions are not justified for lycetol alone because of the simultaneous use of other medication such as magnesia usta and liberal quantities of mineral water. 8.9% of added uric acid was dissolved by lycetol at room temperature as observed by Meisels (1902). However, the nature of the solvent (urine or water) is not stated in the only abstract available. Lycetol has no influence on the excretion of uric acid by birds with experimental gout (Waucomont, 1912). The clinical condition was also uninfluenced.

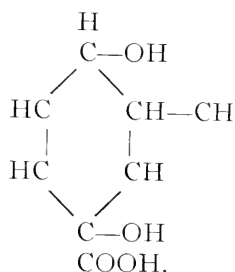
*Sidonal*.—This is a piperazin salt of quinic acid, called piperazin quinate and possesses the following chemical formula



The effects on uric acid or urate should differ in no essential respect from the effects of the individual components, and this seems to be the case according to the reports in the literature. Bardet (1901) administered 3 to 5 gm. of the drug daily to 3 patients and observed its effect on diuresis, uric acid excretion and reaction of urine. It was found that these remained practically uninfluenced. According to Blumenthal (1900) the excretion of uric acid is reduced by 40 to 50% with daily injections of 5 to 8 gm. of the drug, and in place of uric acid, hippuric acid appears in the urine. Mylius (1900) makes a favorable report, unaccompanied by any data, on the use of sidonal in gout. Rosenthal (1900) reports that in a case of gout the administration of 100 gm. of sidonal led to the reduction of uric acid in urine from 0.75 gm. per liter to 0.332 gm. and that the tension of the skin over the tophi and redness disappeared. After a pause of 3 weeks, 100 gm. of sidonal was administered with similar improvement. This was repeated again in 3 months, and 6 months later the condition is said to have remained permanently improved. Klemperer (1900) is dubious about the therapeutic value of sidonal in gout because the value of piperazin has not been demonstrated and, in his experience, quinic acid is also worthless. The experiments of Foerster (1899) showed that the administration of sidonal (to six patients) causes no important differences in the excretion of uric acid. 10 to 25 gm. of the drug were used daily for 3

to 5 days using suitable controls. Foerster could not confirm the reported claims of his day, and pointed out that some other explanation for the reported effects must be sought rather than the drug itself. Meisels (1902) reports that sidonal dissolves 35.5% of added uric acid, but the temperature and solvent used are not stated in the only report available. Schlager (1900) reports such beneficial effects as reduction in uric acid excretion from the administration of sidonal to 6 cases of "uric acid diathesis," increased diuresis, diminished turbidity of urine and lessening of renal pains in an individual with renal stone. Waucomont (1912) observed no beneficial effects on the clinical condition of experimental gout in birds.

*Quinic Acid.*—This is closely related to benzoic and other aromatic acids. Its formula is



It has been recommended as a urate solvent, but its mode of action has remained unexplained. It has recently been shown by Denis (1915) that the administration of quinic acid has little or no effect on the uric acid content of urine or blood, leaving the threshold of the kidney for uric acid unaffected, and differing in this respect from salicylate and atophan. Likewise the information obtained from the literature referred to in this section, indicates that quinic acid possesses no special virtues as a urate solvent.

According to Cushny (1910) quinic acid has no effect whatever on the quantity of uric acid excreted. Foerster (1900) claimed to have demonstrated an increase in uric acid excretion, and urine output following the administration of a total of 50 gm. in several days. Unfortunately the results are complicated by the simultaneous administration of thymus gland. Fraenkel (1912) states that Weiss observed a diminution in uric acid excretion. Hupfer (1903), however, denies the lessening effect on uric acid excretion by quinic acid. The experiments of Meisels (1902) on pigeons with artificial urate deposits seem to indicate that quinic acid is less efficient than sidonal as an inhibitor of the deposits. In human individuals sidonal increased the excretion of uric acid from 0.3289 to 1.968 gm. Taltavall and Gies (1903) found that in dogs on constant diets, quinic acid (1 to 20 gm. in 10 days) did not affect the elimination of uric acid. This agrees with the observations of Hupfer on human individuals. This was further confirmed by Ulrici (1901), who also showed that quinic acid does not influence nitrogenous metabolism.

*Lysidin.*—Chemically this is ethylen-ethylen diamine, and the formula is



Lysidine is said to possess five times the solvent action of piperazin (Fraenkel 1912). According to Goodbody (1896), urine containing 0.1% of the drug increased the solution of urate in normal urine from 0.059% to 0.065%; the acidity was diminished from 0.35% to 0.33%. Following the administration of 1 gm. of lysidine daily for 8 days, and 2 gm. daily for 4 days, maintaining the diet, fluid intake, and exercise constant, Goodbody observed an increase in diuresis, increase in uric acid excretion, but no important changes in urinary reaction. In these particulars lysidine was found to excel piperazin. Has-kins (1916) found that lysidin can act as a uric acid solvent (added uric acid) in acid urine. The urinary reaction may remain truly acid. If the urine is alkaline, when either lysidine or piperazin is administered, its uric acid solvent property is enhanced, but it is not a practical therapeutic agent because if large enough doses are used it is unsafe and possesses no advantage over sodium citrate or bicarbonate. Meisels (1902) observed that lysidin in aqueous solution can dissolve 47.86% of added uric acid. Meisels also observed that the administration of lysidine gave relief to pigeons with urate deposits. According to Neubauer and Vogel, Ladenburg (1894) claims lysidine urate is soluble in 6 parts of water.

Clinically, however, Ortnier (1908) states he cannot recommend lysidine as a urate solvent, adding that "it not only has never done any good in my experience, but once, after a few days' use, it caused an almost general acute, painful, and partly bullous eczema." According to Schmiedeberg (1909) lysidine dissolves uric acid in vitro more easily than lithium carbonate, but the addition of 1% sodium chloride completely inhibits this. Favorable reports on the use of lysidine in gout and urate deposits are made by Wolf (1907). Lysidine is not regarded as an effective calculi solvent by Casper (1897). According to Ortowski (1900) lysidine is inferior to hexamethylenamine as a uric acid solvent. By the addition of uric acid to urine containing lysidine (0.5%), Tun-cliffe and Rosenheim (1908) found the solvent power to be 33.2 when that of urine alone was expressed as 1. The solubility of sodium biurate in beef serum containing lysidine (0.1%) was 1:25,000 as compared with 1:60,000 in serum alone. The solvent power of piperidin was higher, 1:12,000.

*Urol.*—Chemically urol is a urea salt of quinic acid, (2 molecules of urea combining with 1 molecule of quinic acid), and according to Frieser (1902) is beneficial in gout, rendering the urine free of "uric acid excess." However, no data are offered to substantiate this vague claim.

*Urosin (lithium quinate).*—The administration of 10 to 25 gm. daily for 3 to 5 days produced no important differences in the excretion of uric acid, nitrogen and phosphate (Foerster, 1900). Foerster could not confirm the reported claims of other observers, such, for instance, as that of Weiss (1900), who claimed that urosin lessens uric acid formation because of its quinic acid content.

*Salicylate.*—The chemical formula for salicylic acid is  $C_6H_4(OH)(COOH)$ . It has been frequently shown that the administration of salicylate increase the elimination of uric acid, but the mechanism of action has not been thoroughly understood. It has recently been shown by Denis (1915) that the ad-



ministration of salicylate leads to a diminution of uric acid in the blood with a simultaneous increase in the uric acid content of the urine, and this is interpreted as due to a lowering of the threshold of the kidney for uric acid excretion. The earlier literature has been abstracted in a previous contribution (Hanzlik, 1915) and need not be further detailed here.

*Phenyl-quinoline.*—The effects of a number of phenyl-quinolines on uric acid excretion were studied by Luzzatto and Ciusa (1913) with negative results. The most favorable results were obtained with diphenyl-quinoline carboxylic acid (atophan) and diapurin ( $\alpha$ -phenyl- $\beta$ -naphthoquinoline carboxylic acid).

It would be altogether out of place here to refer to the rather extensive literature on atophan. Other sources (Sollmann, 1917; Fraenkel, 1912) may be consulted for this. Briefly, however, it may be stated that atophan is related chemically and pharmacologically to salicylic acid. For lack of any other explanation it was generally held that atophan is a "mobilizer" of uric acid in the tissues. However, it has been shown by Folin and Lyman (1913) and Fine and Chace (1914) that the effects of atophan on the excretion of uric acid consists of an increased elimination in the urine with simultaneous decrease in the uric acid content of the blood. Haskins (1913) also observed that atophan increases the elimination of uric acid in the urine.

*Colchicum.*—This was found by Rockwood and Van Epps (1900) and Abl (1913) to decrease uric acid excretion. On the other hand, an increase in excretion was reported by Jackson and Blackfan (1907). Most recently, however, it has been shown by Denis (1915) that the administration of wine of colchicum exerts little or no influence on the uric acid content of the blood and urine.

*Piperidin.\**—Tunicliffe (1897) found 50 c.c. of a 10% solution to completely dissolve 10 gm. of uric acid. The piperidin urate could be completely precipitated by sodium chloride and its solubility was found to be 5.3% and 25% at 17° and 36°, respectively. Aqueous solutions possessed an alkaline reaction. According to Tunicliffe his results do not confirm the high solvent power ascribed to piperidin urate by others.

#### 14. SUMMARY.

There is no reliable evidence to show that piperazin, in small or therapeutic doses, imparts to urine urate solvent qualities, either by direct addition or after excretion.

Excessive doses of the drug produce a slight but practically negligible increase in uric acid excretion, the same being effectively secured by the use of such well known alkalis as bicarbonate and citrate.

The solvent action of low concentrations of piperazin on calculi is practically negligible. In very high concentrations a solvent power, though limited and doubtful, seems to exist.

There is no reliable evidence to indicate that piperazin can prevent or remove urate deposits.

\*For description see foot note, Section 4.

Diuresis is uninfluenced by the administration of even large doses of piperazin.

The direct addition of piperazin to urine renders it alkaline. However, after administration the reaction remains unchanged because in its passage though the body enough piperazin is destroyed to markedly reduce its concentration in the urine.

Scientific evidence, though limited, and clinical opinion indicate that piperazin is valueless in gout.

The administration of piperazin may be attended with serious side actions.

There is sufficient scientific evidence to indicate the worthlessness of the following as urate solvents; urosin, lycetol, sidonal, quinic acid, lysidine, urol, quinoline, colchicum and piperidin.

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## CHRONIC COLITIS AND ITS ROENTGENOLOGIC FINDINGS\*

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**T**HIS paper comprises a study of a group of cases of chronic colitis in which marked pathologic changes occur in the intestinal wall resulting in inflammatory thickening, contraction, granulation, or ulceration.

Chronic colitis is a disease that is constantly attracting more attention from both the medical and the surgical members of the profession. The number of patients affected with the disease is increasing, probably because the number of cures do not keep pace with the annual incidence of the disease. We have recently reviewed the cases of chronic colitis of this type in the Mayo Clinic in which a roentgen examination of the colon was made, and we wish to submit a résumé of the subject including certain roentgen findings that we believe are of particular importance and have not been sufficiently emphasized (Fig. 1).

The etiology in these cases of colitis is obscure. It is probably due to some constitutional derangement with a low grade of intoxication that seems to affect primarily the mucous lining of the large intestine, and at times, the terminal portion of the ileum. Chronic constipation probably plays a part as a predisposing factor but does not explain all. It may be due primarily to some specific organism upon which is superimposed considerable localized trauma, local irritation, and mixed infections. The rectum and sigmoid are first and always the most severely affected but eventually the process tends to become diffuse throughout the entire colon and occasionally the lower 12 to 24 inches of the terminal ileum. The early changes occur in the mucosa, which becomes reddened, thickened, and infiltrated with serum and blood cells. This chronic inflammatory reaction results in the formation of granulation tissue. The mucous glands are increased in number and hypertrophied, or they may become atrophic, small or cystic. The mucous folds are increased or decreased in number and lose their normal appearance. There is an overproduction and hypersecretion of mucus. Polypi and pedunculated papillomas may be formed. Localized superficial erosions and chronic ulcerations are frequently superimposed on all these changes, especially when there are polypi and papillomas, giving rise to deeper mixed infection and more profuse bleeding. In the severe and long standing cases localized abscesses and extensive tissue destruction of the mucosa and the submucosa complicate the disease. The remaining structures of the bowel wall undergo corresponding inflammatory changes and they may occur quite early. There is always an edema and lymphocytic infiltration and exudation in the other coats of the intestinal wall accompanying the changes in the mucosa. The coats are congested and thickened, multiple minute areas of scar tissue are formed, the bowel is contracted down, and is comparatively less yielding and less flexible (Fig. 2). Invariably the serosa is covered with a

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fibrinoplastic lymph exudate and becomes devoid of its normal luster. Frequently the peritoneal cavity contains a small amount of fluid and lymph.

The symptomatology is quite well known. There is usually a history of sea-



Fig. 1. (106151.) Normal colon.

sonal spells of chronic diarrhea and spurious diarrhea, with five to twenty, or more, stools per day over a period of weeks or months at a time for many years. The stools usually are thin and watery and contain much mucus, occasionally purulent material, and at times, blood in varying amounts from a trace to

moderate or profuse quantities, depending on the duration and the severity of the disease. There may be traces of partially undigested food particles in the stools. There are intervals of complete or partial relief, during which time constipation may be present or the symptoms may be mild and more or less



Fig. 2.—(126013.) Female, age 25 years. Chronic colitis of 8 years' duration. Roentgenogram shows a small smooth colon, with absence of haustra especially well marked from the hepatic flexure onward. Ileocecal valve incompetent.

continuous, with a history of acute exacerbations from time to time. The use of the coarser and irritating foods usually exaggerates the condition, and dietetic indiscretions often precipitate an attack. Pain and tenderness are often absent. When present they vary from a diffuse or localized abdominal soreness on pressure, to severe tenderness and marked muscular rigidity. The left lower quadrant

of the abdomen is the area most commonly affected, but this condition may be present over the course of the entire colon. Rectal tenesmus and pain at defecation are often distressing symptoms.

Loss of weight is common during severe attacks, but patients usually regain



Fig. 3.—(107128.) Male, age 54. Clinical diagnosis of chronic colitis of eleven years' duration. Roentgenogram shows a narrow, smooth, sausage-like colon. Both flexures posed. Incompetent ileocecal valve. No haustration seen.

in the intervals. In some cases the patient is surprisingly well nourished and well developed. Nausea and vomiting are unusual and the appetite is ordinarily very good. An acute attack may prove fatal in a few weeks or months from prostration, dessication, repeated intestinal hemorrhages, or from perforation of the bowel with general peritonitis.

In the event of other pronounced constitutional symptoms, or of the stools containing much blood, there is a moderate or severe grade of secondary anemia. There may be a slight leucocytosis, and in the presence of tissue destruction and mixed infection, there is always present an increase in the white blood count with a moderate predominance of polynuclear cells. Eosinophilia is rare. Digital examination of the rectum usually reveals a roughened and granular mucosa which is hypersensitive, painful, and bleeds easily. The anal sphincter is usually very spastic. The disease is resistant to almost all forms of medical treatment, although palliative measures may control the symptoms to a large extent for long periods of time. Spontaneous remissions are common in the milder stages and forms of the disease.

The incidence of the disease is slightly larger in males than in females, but there is very little difference, and the disease runs about the same clinical course in both sexes. Rural people and those living in the smaller towns appear to be more commonly affected. There seems to be no particular geographical distribution of the cases examined in the Mayo Clinic.

Repeated stool examinations are always essential as it is necessary to rule out the more common causes of chronic diarrhea. In these cases no intestinal parasites are found. It must be remembered, however, that in an entamebic colitis that has been treated with emetin, the stools may be free from parasites on several examinations and still some of the symptoms persist. Theoretically tuberculous and other specific bacterial infections can be identified by centrifugation of a small portion of the stool content and microscopic examination of properly stained smears. There is also usually a suggestive history and other symptoms and physical signs present in tuberculous and bacillary colitis cases. A history of luetic infection, other constitutional stigmata and the Wassermann reaction will put one on guard for the possibility of a syphilitic lesion in the lower part of the intestinal tract, although, of course, both conditions may be present. Fortunately syphilis of the rectum and colon is very rare.

The proctoscopic examination is an efficient aid in the differential diagnosis, and in ruling out a low-lying malignancy, either alone or superimposed on some other chronic process. Proctoscopic examination usually reveals a chronic indurative proctitis and sigmoiditis of a granular type which bleeds easily with few ulcerations irregularly distributed. Occasionally small polypi or papillomas may be seen. Frequently it is only by removing a small piece of suspicious tissue and subjecting it to careful microscopic examination that malignancy and the infectious granulomas of the rectum can reasonably be excluded.

Very little has been written concerning the signs elicited in the roentgen examination of chronic colitis, and no great importance has been attached to roentgenologic findings in this disease up to this time. In 1912 Schwarz,<sup>1</sup> of Vienna, described the characteristic features in a small number of cases of chronic colitis. Among other things he included the phenomena of a small and smooth bowel, absence of haustration and incompetence of the ileocecal valve (Fig. 3). We have found from an examination of a number of colons in chronic colitis that these conditions are invariably present and we believe that their importance should be emphasized. These roentgenologic phenomena are not due to spasm,



but result from the organic alterations in the bowel wall, because definite pathologic changes are found in both the gross and the microscopic appearance of all the coats. Furthermore, they are permanent and cannot be altered in their main appearances and characteristics by the administration of antispasmodics to physio-



Fig. 4.—(12716.) Female, age 57 years. History of chronic constipation and migraine for a period of twenty-five years. Roentgenogram shows marked redundancy of the colon. Incompetent ileocecal valve. The bowel is smooth and its outline is practically without haustration, but the colon is not contracted. Further the history distinguishes the case from a chronic colitis.

logic effect prior to a second roentgen examination. Besides, enterospasm has not been observed by us during the roentgen examination in this type of colitis.

There are certain other conditions of the large bowel in which some of these phenomena may also be found to a varying degree; but, as a rule, they can be

eliminated either by a correlation of the roentgen findings with the clinical data, or the roentgen picture alone may definitely indicate a lesion other than colitis. For example, in an occasional case of chronic constipation, the large bowel may be smooth and show no haustration (Fig. 4). Exceptionally also, in cancer of



Fig. 5.—(144665.) Female, age 44 years. Filling defect in the right half of the transverse colon. Roentgen diagnosis: Carcinoma. The remaining bowel is smooth and unhastrated, but not contracted. The filling defect was produced by a carcinoma, as proved at operation.

the colon, the unaffected portion of the bowel may have smooth, unhastrated borders (Fig. 5). However, these findings are not constant. In such instances the lack of haustration is doubtless due to relaxation of the longitudinal muscle bands—the tenia.

The incompetence of the ileocecal valve, which has been noted rather constantly in cases of chronic colitis, is not of itself diagnostically important, since we find it in a very high percentage of all cases examined, whether normal or pathologic. But, in conjunction with other roentgen signs of chronic colitis, it may have some corroborative value, when the barium runs into the ileum spontaneously and profusely.

It is worthy of note that in a limited number of cases examined of chronic endamebic colitis, we did not find a roentgenologic picture in any measure differing from the normal. The group included cases of long standing and of recent exacerbations but none that were prostrated. Sanford<sup>2</sup> has emphasized the fact that the entire syndrome of chronic endamebic colitis as seen in the North is not so severe as in the South and the tropics, which may explain the absence of marked pathologic changes in the bowel wall. However that may be, we believe that the roentgen examination offers distinct value in the differentiation of these two diseases, especially as seen in this latitude.

Our own experience suggests that the roentgen findings when carefully correlated with the clinical history can be made of much use in the diagnosis of chronic colitis. The roentgenogram also enables a more accurate estimate of the extent of involvement and thus may serve as a guide in determining whether the treatment shall be medical or surgical.

Both roentgenoscopy and roentgenography were employed in the cases herewith reported. The enema was preferred to the ingested meal. In an extensive chronic colitis of long standing the capacity of the colon is materially lessened. It fills very rapidly, and if the clyster is administered with much force the patient is very likely to expel it before the roentgen observations are completed.

The following illustrative cases were selected from the group studied:

**Case 146817.**—Male, aged 38; shoemaker. Registered November 30, 1915.

*Precious History.*—Pneumonia April, 1915.

*Present Illness.*—For 2 years spells of diarrhea with 15 to 20 stools per day of a thin watery character, with much mucus and at times blood stained; odor not offensive. With attacks there was loss of weight and strength. During the past four months, interval of but two weeks when he was free from severe symptoms, and during the past three months there has been considerable bright red blood in the stools, much abdominal cramp-like colic and rectal tenesmus. Never any chills or fever noted.

*Physical Examination.*—Considerable emaciation. Evidence of moderate anemia. Pyorrhea alveolaris and gingivitis present. Palpable liver margin. Slight tympanitis and slight diffuse abdominal tenderness. Digital examination of rectum negative.

*Urinalysis.*—Negative.

*Blood Examination.*—Hemoglobin 70 per cent; 4,520,000 red blood cells; 10,000 white blood cells; 38 per cent polymorphonuclear leucocytes; 44.3 small lymphocytes; 16.7 per cent large lymphocytes; 1.0 per cent eosinophiles. Wassermann, negative.

*Stool Examination.*—Red blood cells and pus cells present, no parasites found. Culture from stool negative for Shiga bacilli.

*Roentgen Examination.*—Colon, small and smooth throughout. No visible haustration. Ileocecal valve, incompetent. (Fig. 6.)

*Clinical Diagnosis.*—Chronic colitis; pyorrhea alveolaris and gingivitis.

*Operative Findings.*—Colon contracted to about caliber of small intestine, and presents appearance of a chronic inflammatory reaction with thickened and edematous walls, increasing in severity from cecum downward to rectum.

*Operation.*—Heostomy (Brown)—Appendectomy secondary.

**Case 134997.**—Female, aged 33. Housewife. Registered September 13, 1915.

*Previous History.*—Negative.

*Present Illness.*—A 9 year history of chronic constipation with passage of some mucus in the stools, and at times blood tinged. For the past 2 years constipation has been more marked, requiring brisk laxatives or purgatives which in turn caused a diarrhea that re-



Fig. 6.—(146817.)

quired medicine to control. For the past 6 months she has complained of diarrhea and spurious diarrhea associated with the passing of mucus streaked with bright red blood, also cramp-like abdominal pains and rectal tenesmus. Normal weight 107 pounds, present weight 97 pounds.

*Physical Examination.*—Thin and pale in appearance. Marked intestinal peristalsis demonstrable. Digital examination of the rectum painful; rectal mucosa roughened and congested.

*Urinalysis.*—Negative.

*Blood Examination.*—Hemoglobin 60 per cent, 4,500,000 red blood cells, 14,400 white blood cells, and a normal differential count. The Wassermann was negative.

*Stool Examination.*—Red blood cells and pus cells present, no parasites found.

*Proctoscopic Examination.*—Chronic proctitis and sigmoiditis with some superficial ulcerations.



Fig. 7. (134997.)

*Roentgen Examination.*—The left arm of the transverse colon, the splenic flexure, the descending colon, and the sigmoid are small, smooth, and unobstructed. This condition was unchanged after the administration of an antispasmodic to physiologic effect. Note that the disease is limited to the left half of the large bowel as shown in the roentgenogram (Fig. 7).

*Clinical Diagnosis.*—Chronic ulcerative colitis.

*Operative Findings.*—Chronic ulcerative colitis.

*Operation.*—Hleostomy (Brown) Appendectomy secondary.

**Case 160059.**—Female, aged 28. Clerk. Registered May 18, 1916.

*Previous History.*—Negative except appendectomy 10 years ago elsewhere.

*Present Illness.*—For 12 years she had had spells of diarrhea and bloody stools associated with loss of weight and strength. Some temporary relief with medical, dietetic, and local treatment. She has been without symptoms as long as a year at a time. At the present time there are from 6 to 8 stools daily, containing much blood and mucus and associated with considerable tenesmus.

*Physical Examination.*—Negative except for some tenderness over the entire colon and considerable tenderness on digital examination of rectum.

*Urinalysis.*—Negative.



Fig. 8.—(160059.)

*Blood Examination.*—Hemoglobin 80 per cent.

*Stool Examination.*—Negative for parasites.

*Proctoscopic Examination.*—Examination very difficult on account of the contracted condition of the rectum. Marked proctitis which bleeds easily. No gross ulcerations. The sigmoid could not be examined on account of spasm.

*Röntgen Examination.*—Beginning at the hepatic flexure the colon was smooth, contracted and without haustral markings. The narrowing was most marked in the left half of the transverse colon, the descending colon and the sigmoid. The transverse colon was redundant. Ileocecal valve incompetent. (Fig. 8.)

*Clinical Diagnosis.*—Chronic granular colitis.

*Operative Findings.*—Granular type of chronic colitis. Definite thickening and nar-

rowing of the colon wall. Slight injection of its blood vessels. Appendix removed at former operation elsewhere.

*Operation.*—Ileostomy (Brown).

*Autopsy.*—General peritonitis. Colon showed chronic inflammatory changes with marked thickening throughout its entire wall and many petechial hemorrhages, old and recent, in its mucosa. No ulcerations. Small papilloma in the descending colon.



Fig. 9. (172507.)

**Case 172507.**—Male, aged 35. Iowa farmer. Registered September 14, 1916.

*Precious History.*—Negative.

*Present Illness.*—For 3 years there have been spells of diarrhea with blood stained mucus in the stools, and associated with rectal tenesmus. The early spells were of about 6 weeks duration. Considerable loss of weight with each attack. During the past 14 months the symptoms have been practically continuous and the stools contained considerable blood. For

the bleeding he had a cauterization of the rectal mucosa elsewhere with practically no improvement. For the 3 weeks prior to admission to the clinic he had had 10 to 20 stools per day, containing much blood and mucus. History of slight fever at times during past month.

*Physical Examination.*—Twenty pounds loss of weight. Evidence of anemia. Slightly tender over entire abdomen. Digital examination of rectum painful and difficult.

*Urinalysis.*—Negative.

*Blood Examination.*—Marked secondary anemia with an average hemoglobin of 34 per cent. Wassermann negative.

*Stool Examination.*—Negative for parasites; red blood cells and pus cells present.

*Proctoscopic Examination.*—External and internal hemorrhoids were found but not sufficient to account for all symptoms. Large fissure into rectum. Indurated area in anterior wall of rectum 2 inches up.

*Roentgen Examination.*—Roentgenoscopy.—Entire colon smooth, contracted and without haustration. Roentgenogram (after involuntary partial evacuation).—Hepatic flexure, descending colon and sigmoid mottled in appearance; ilcoecal valve incompetent (Fig. 9).

*Clinical Diagnosis.*—Fissure of rectum (postoperative), hemorrhoids; chronic ulcerative colitis; secondary anemia.

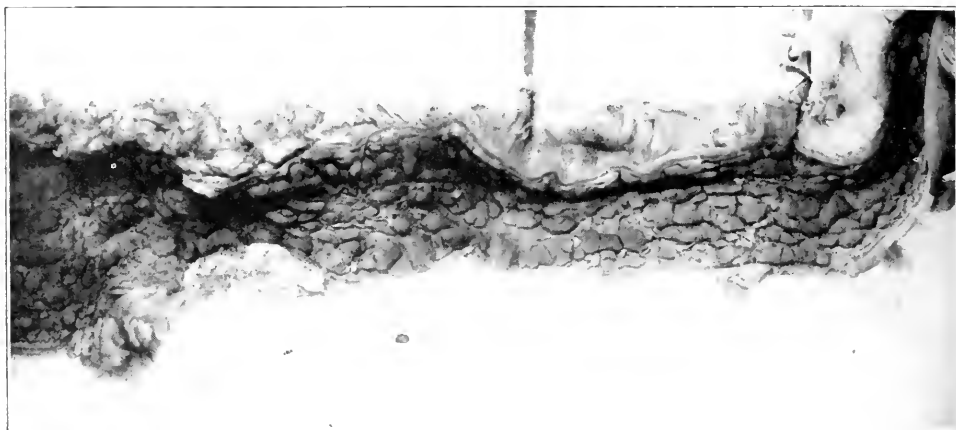


Fig. 10.—(172507.)

*Clinical Notations.*—Medical and dietetic treatment, and blood transfusion without improvement. Died 13 days after admission.

*Autopsy.*—Chronic inflammatory changes in the wall of the large bowel with marked diffuse edema and thickening of all its coats. The mucosa shows chronic ulcerations in the lower colon and rectum (Fig. 10).

#### CONCLUSIONS.

1. There is a group or chain of subjective and objective symptoms that is quite characteristic of chronic colitis.

2. There are definite organic changes in all the coats of the colon wall in chronic colitis, resulting from chronic inflammatory reaction with edema, lymphocytic infiltration, thickening, scar tissue formation, and contraction.

3. The roentgen examination in these cases shows the colon to be small, smooth and without haustration in the part or parts affected.

4. A more accurate estimate as to the extent and severity of the involvement can be obtained by a correlation of the clinical history with the roentgen findings.



5. The roentgenogram will frequently be an aid in determining the course of subsequent treatment.

6. In a limited number of examinations we have not found cases of chronic endamebic colitis which furnish any characteristic or similar roentgen findings. Therefore, it appears to be an aid in differentiating amebic from chronic colitis.

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## CERTAIN ASPECTS OF PURULENT MENINGITIS\*

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**E**PIDEMIC purulent meningitis due to infection with the diplococcus of Weichselbaum rarely offers great difficulty of diagnosis, and the acute purulent meningitis secondary to obvious infection of the middle ear or the air sinuses is as a rule recognized early and with ease. There are, however, certain cases such as the three which came to autopsy in this hospital but a few weeks apart which are in a different category. They present points of interest in etiology and in diagnosis since in these instances only one was recognized. The second and third were diagnosed, respectively, uremia and cerebral hemorrhage though the former showed only a moderately pathological kidney and the latter no trace of hemorrhage in the brain.

Oppenheim<sup>1</sup> discussing the etiology of this variety of meningitis names as reported etiological factors: streptococcus, staphylococcus, pneumococcus, pneumobacillus, bacillus typhosus, bacterium coli, bacillus lactis aerogenes and the bacillus influenzae. The primary source of infection may be any acute infection, notably pneumonia, typhoid, ulcerative endocarditis, variola, acute rheumatic fever, influenza and gonorrhea, (?) or the condition may complicate a septicemia or a pyemia.

The clinical history and autopsy protocol are in abstract.

J. B., Hospital No. A-6688, was a white male machinist, aged 35. He was admitted to the hospital on October 8, 1916, complaining of pain in the right side on deep inspiration, chills and fever. The family history was negative. The patient denied the usual infections of childhood but admitted chancre at 20 and gonorrheal urethritis at 22. He averaged five glasses of whiskey and two of beer daily.

One week before admission he spent a day in the rain fishing. Five days later there was an acute onset with fever, chills, and pain in the right side on deep inspiration. About 24 hours later he began to cough up a brownish sputum. He sweated at night.

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On admission he was obstreperous and therefore in shackles. The chest was symmetrical and expansion was equal on the two sides but was rapid and labored and the patient was rather cyanotic. Vocal fremitus was increased over the right side of the back except at the extreme base where it was diminished. On percussion there was dullness over the right back and in the axilla. A few moist râles could be heard over the right back, and at the extreme base respiration was distant. The heart and abdomen are noted as clinically negative. The cervical glands were palpable and firm. There was marked pyorrhea alveolaris. The urine, except for a few hyaline and granular casts, was normal. The white count was 16,800.

Neurological examination showed moderately dilated pupils which reacted to both light and accommodation. The external ocular muscles functionated normally. The neck was not rigid. "The reflexes are normal—there is slight tremor" (of the hands?).

The history notes that the patient's delirium continued and that on the tenth day after admission he began to vomit and showed a suggestion of rigidity of the neck. On the seventeenth day after admission a radiograph of the chest was negative except for some slight clouding at the apices. On the eighteenth day paracentesis of the pericardium was attempted without obtaining fluid and on the same day lumbar puncture liberated 40 c.c. of cloudy fluid "under pressure." Cultures showed an organism which was "either the pneumococcus or the streptococcus viridans." During the period of hospital residence the temperature ranged between 100° and 102° F. and the pulse was slow in proportion (74 to 90) during the first week. The patient died on the twenty-first day after admission.

*Clinical Diagnosis.*—Pneumonia; cerebrospinal meningitis; chronic diffuse nephritis; pericarditis (?); acute endocarditis.

#### AUTOPSY PROTOCOL. (M. G.)

There was a paracentesis wound in the fifth costal interspace. Opening the pericardium liberated about 5 ounces of bloody fluid which came from a wound of the heart wall about one inch from the apex. The left side of the heart was hypertrophied and the musculature was generally edematous. There was a large mass of vegetations on the aortic cusps and surrounding cardiac muscle. The liver, spleen and kidneys were all congested and edematous and showed multiple infarcts. Very fine pleural adhesions prevented the collapse of the lungs on opening the thorax, but there was no excess of pleural fluid. The organs were edematous and of brick red color on section with areas of anthracosis. There were scars of old adhesions on the lower and middle right lobes. Neither lung was consolidated but crepitated throughout. The gastric mucosa was congested and edematous and the veins about the cardia much injected.

On removing the calvarium the brain was found to be bathed in pus and the cerebrospinal fluid purulent.

*Anatomical Diagnosis.*—Diffuse purulent meningitis; edema and congestion of liver, spleen and kidneys and gastrointestinal mucosa; pleuritic adhe-

sions; hypertrophy of left heart; acute vegetative aortic valvular endocarditis; cardiac apical wound with hemorrhage into the pericardial sac; acute splenic tumor; infarct of the liver and multiple infarcts of spleen and kidneys.

No focus of infection was found in the skull and it seems fair to trace it back to the aortic vegetations or even back to the pyorrhea. The multiple infarcts may well have been the result of infection. Without violating probability one may reconstruct the history as follows: pyorrheal infection at a remote date involving ultimately the liver, spleen, kidneys, and endocardium, and perhaps the pleura. Chronic alcoholic poisoning plus a day of exposure broke down the resistance and the infection became general. The very definite clinical signs in the lungs with rather inconsiderable pathological findings remains unclear. At what time the central nervous system became involved is doubtful. Delirium and fever coincided from the first, but in an alcoholic who presented such a classical picture of pneumonia the central nervous system was rather likely to escape attention. It is regrettable that lumbar puncture was not done before the onset of definite meningeal symptoms as either negative or positive findings would be an addition to the postmortem study of the case.

The differential diagnosis of acute purulent meningitis offers some difficulties particularly in the early stages when fever is not infrequently absent. Osler<sup>2</sup> reports the following case as typical of this difficulty. I have taken the liberty of abbreviating.

Male, aged 57. Had been drinking heavily and when admitted was delirious. His friends stated that he had been in this condition for a week. His temperature on admission was  $97.5^{\circ}$  and there was no symptom of meningitis. The case was regarded as delirium tremens until the fourth day when his temperature rose suddenly to  $105^{\circ}$ . He died two days later. Necropsy showed extensive yellowish purulent exudate over the hemispheres, turbid exudate at the base and along the Sylvian fissures and in excess in the ventricles. Direct smears showed a rounded coccus but the cultures yielded a diplobacillus. From the lungs which showed a slight bronchopneumonia, the diplococcus of pneumonia was recovered.

It is a platitude to remark that gastrointestinal conditions can produce in children a complex hardly distinguishable from meningitis, the so-called menin-gismus. The "wet brain,"—"serous meningitis,"—of alcoholics, which is probably an edema of the brain pure and simple, can simulate the febrile period of true meningitis accurately. Perhaps one would better say that the early stage of a meningitis which ultimately becomes purulent is serous. Luetic meningitis may occasionally have a course so acute as to give rise to confusion.<sup>3</sup> Major hysteria can, of course, simulate any disease one may discuss. Uremia is mentioned by most writers as capable of giving rise to difficulty.<sup>1, 4, 5</sup> However, all these conditions can be accurately differentiated by lumbar puncture and urinalysis. A mistake could have been avoided by this means in the following case.

The clinical history is brief and can be given in full. "The patient was admitted to the ward in a dying condition. Breathing stertorous. Pulse very feeble and irregular. The heart could not be heard owing to large râles over the chest. Eyes rolled upward and to the left. The patient had been catheterized

in the receiving ward and the urine found to contain much albumin. Coma was profound. Reflexes were abolished. The patient did not respond to stimulation. He died an hour after admission to the ward."

In abstract, the postmortem findings were as follows: The skull showed the evidence of an old trepanation—a lack of continuity about 5 cm. in diameter in the left temporal region. The dura was more than ordinarily adherent to both the skull and the pia, particularly along the longitudinal fissure. The brain showed the usual picture of acute purulent meningitis. The greatest accumulations of pus were over the parietal cortex of the vault and both the inferior and superior aspect of the frontal lobes, over the inner aspect of the temporal lobes, in the cisternæ and along the base of the cerebellum. The pituitary was almost completely destroyed by suppuration. Other than edema or cloudiness of the membranes of the posterior nares and the sphenoidal sinus, no focus of infection could be found in the head.

In the right hypogastrium there was an old apparently surgical scar through which hernia had occurred. The appendix was missing. The liver, spleen, stomach and intestines showed no important pathological change. The right heart was hypertrophied, the coronary vessels dilated as also to a slight extent the pulmonary and tricuspid orifices. There was hypertrophy of the ventricular wall and moderate (30 c.c.) effusion in the pericardium. The lungs showed some old fibrous pleural adhesions and marked edema. The kidneys were large and their capsule stripped with difficulty. The cut surface was pale and the cortex marked with lines of interlobular congestion. The cortex was thick and not well delimited from the medulla. There was slight papillary fibrosis. The brain showed the usual picture of acute purulent meningitis. Smears showed a mixed infection probably as a result of postmortem invasion—a large spore bearing bacillus, a small diplococcus (?) which was not intracellular, a slender polar stained bacillus and a rod-like form resembling the bacillus influenzae. All were Gram-negative.

In this case the clinical diagnosis was uremia, yet postmortem there was not sufficient renal change to verify it. Lumbar puncture would have definitely settled this diagnosis.

A third case is of little clinical interest because of the inadequate examination and history and the entirely unwarranted diagnosis. Pathologically the case presents an unusual distribution.

Again the brevity of the history permits its presentation in full. "Admitted to the ward on a stretcher in a semi-comatose condition. Pupils reacted to light very little: conjunctival reflex absent: reflexes of the lower extremities present on both sides. No abdominal; no cremasteric reflex. No apparent difference in both extremities. Pulse good; heart normal; temperature 104°."

The clinical diagnosis was cerebral hemorrhage! In the abstract the autopsy protocol is as follows: The body of a well developed fairly obese negro of about 50 years. Pupils equal and moderately dilated. Marked pyorrhea alveolaris and slight evidence of herpes on the upper lip. There were old scars on both tibiæ. The apex of the right lung was adherent to the pleura, but there was no pleural effusion.

There was a slight increase of sub-epicardial fat and of pericardial fluid. The heart was somewhat enlarged, the right side hypertrophied and the musculature fibrotic. The mitral and tricuspid valves were somewhat thickened but like the other two were entirely competent. The aorta was dilated and contained fatty plaques with one area of calcification. The course of the (abdominal)? aorta showed multiple areas of subintimal hyaline thickening. The coronaries were somewhat tortuous but their ostia were patent. In the substance of the liver were numerous yellowish-white patches. The kidneys showed the usual picture of chronic interstitial fibrosis and some retention cysts. The spleen and pancreas were congested. The stomach was enormously distended and the superficial veins enlarged particularly at the cardiac end. There too the mucous membrane was tremendously congested. Other than a solitary petechial hemorrhage the mucous membrane of the intestines showed no im-

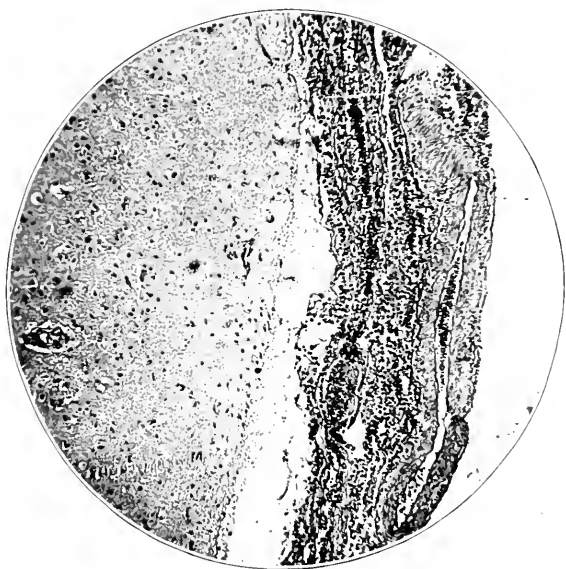


Fig. 1.

portant change but there were dense adhesions of the upper loops to themselves and of the sigmoid to itself and to the descending colon. There was moderate inflammation of the tip of the appendix.

When the calvarium was removed the dura was not unusually adherent. The basilar vessels were not sclerotic but there was marked cerebral congestion. The pia was generally edematous and showed multiple areas of opacity resembling exactly those seen in luetic meningitis, but microscopic section through these showed an acute purulent reaction. (Fig. 1.) Careful examination by coronal sections showed no trace of hemorrhage. There was no evidence of skull fracture.

The grounds for the diagnosis seem insecure. The patient lived twenty-four hours but no history was secured and the blood pressure is not recorded. There is no mention of hemiplegia or any other form of paralysis. In the

face of this and of the high temperature the diagnosis of hemorrhage is unwarranted. Cultures of the spinal fluid would have been of exceptional interest. Whether such slight degrees of inflammation could be detected in this way deserves investigation. It is to be noted that here too there was a marked pyorrhea and as in the first case this may have been the original source of infection.

All three of the writer's cases and that quoted from Osler came into medical hands in an abnormal mental state,—two in delirium and two with marked clouding of consciousness. Unless definitely contraindicated it would seem almost imperative to perform lumbar puncture on patients so received. Osler in the lecture quoted says: "During the past ten years no single measure of greater value in diagnosis has been introduced than Quincke's lumbar puncture." Of the three cases personally reported here, in the first an earlier puncture would have prevented the disastrous attempt at paracentesis of the pericardium: in the second, it would have corrected an absolutely erroneous diagnosis.

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# LABORATORY METHODS

## APPARATUS FOR USE IN EXAMINING FECES FOR EVIDENCES OF PARASITISM\*

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IN a previous paper,<sup>1</sup> later reissued with addenda,<sup>2</sup> the writer has given a comparative study of methods of examining feces for evidences of parasitism. In that study, the various methods which had been advocated were described and it was pointed out that these numerous modifications of technic had as their object the concentration of parasite eggs and the facilitation of the detection of the eggs or of other parasitic material, such as segments, entire worms, etc. Such concentration is obtained by taking advantage of the differences in physical, chemical, and biological properties between parasitic and nonparasitic material. These are mostly differences in specific gravity, size, physical, and chemical solubility, adhesiveness, and capacity for growth and development.

In comparative tests, the writer found the most satisfactory procedure to lie in the middle course between the old and crude smear method and the rather elaborate refinements which call in the use of various chemicals. Very dependable results were secured within a quite reasonable time by taking advantage of the purely physical differences existing between parasitic and nonparasitic material. These results were obtained by the use of screens to remove coarse material and use of a liberal amount of water to remove soluble coloring matters and fine matter in suspension. The use of some apparatus for breaking up fecal masses was advocated, and also the use of a centrifuge to save the time required for sedimentation of samples.

In the years since these studies were published, the writer has continued to find such a method as that outlined above quite satisfactory, but has found certain changes in the apparatus advantageous. The method I am using at present is as follows:

Rather large fecal samples, 2 or 3 ounces, are used wherever possible, instead of the classical sample, "about the size of a walnut." The reason for this is that in a general way it is desirable in our work to ascertain whether any parasites are present, not merely whether there is a massive infestation. By using large samples and concentrating the eggs present, it is possible to detect very light infestations. The use of the much simpler smear method, taking a small amount of fecal matter on the end of a toothpick and smearing it on a slide with a drop of water, will perhaps serve the purposes of the busy practitioner who cannot take the time and trouble to use more accurate methods, but it should be understood that the chance of detecting light infestations in this

\*From the Research Laboratory, Parke, Davis and Company, Detroit, Mich.

<sup>1</sup>Bu. Anim. Indust. Bull. 135, 1911, 36 pp.

<sup>2</sup>Idem, with addenda, 1912, 42 pp.

way is much less than where the more elaborate methods are used. Where a physician is primarily concerned with detection of massive infestation with hookworm, the smear method will probably serve very well, especially since there will be clinical indications of such infestations in nearly all cases. But even here it should be noted that in communities where there are massive infestations with hookworm, there will usually be simple or combined infestations with ascarids, whipworms, *Strongyloides* or tapeworm also present, and it is desirable to detect these even in light infestations. Ascarids are regarded as rather innocuous by some physicians, but the writer would prefer an infestation with a moderate amount of hookworms to an infestation with ascarids in any amount from one up. The large size and wandering habits of these worms make them a potential source of serious danger. Cases have been reported where these worms have entered the ducts of the liver and pancreas, crawled up the esophagus and down the trachea to the lungs, or through the eustachian tube and out of the ear; where a single worm has caused enterospasm necessitating operative relief; where they have perforated the intestine and caused peritonitis; and even the not uncommon occurrence of vomiting one or more of these worms, which are about the size of a lead pencil, is a prospect one might well face with hesitancy. In view of these facts it would seem as well worth while to employ means to ascertain with the greatest possible accuracy whether worms were present, as to employ the utmost accuracy to ascertain whether *Plasmodium* or *Treponema* or *gonococcus* or *B. tuberculosis* were present. In any case accurate information is the thing sought.

Large fecal samples are also desirable for the reason that tapeworm infestation is not uncommonly present without eggs being present in the feces. It occasionally happens that segments of tapeworms are present and can be detected by using large samples and screening, and that the same fecal sample will show no tapeworm eggs owing to the fact that the segments have not ruptured and released the eggs. This is less apt to be the case for infestation with *Tenia*, owing to the position of the uterus in that species, though it is a possibility there also, but is not uncommonly the case for such cestodes as *Dipylidium*, where the eggs are retained in egg capsules in the parenchyma of the segment. In addition to tapeworm segments, the screens will occasionally retain entire nematodes or cestodes which are being passed, and of course the same would be true for flukes.

In the method previously given for fecal examinations, the writer described the use of a "milk shaker," of the type where the glasses are shaken up and down, for use in breaking up fecal samples. This apparatus, motor-driven, was very satisfactory in most respects, but had the disadvantage of being very noisy. A substitute for this machine was found in the newer type of mixer, in which a small motor drives a vertical shaft terminating at the bottom in a smooth-edged hexagonal disk. In this machine, which is shown at the right of Fig. 1, the motor head is lowered so that the disk is near the bottom of the inside of the bottle containing the feces, the current automatically turning on and spinning the shaft and disk as the motor head is lowered. This machine seems to be an improvement over the old type for breaking up feces. It works more



rapidly and is practically noiseless. It does not damage nematodes or isolated tapeworm segments which may be present, though it does sometimes break tapeworm strobilæ which may be in the feces. For the purpose of breaking feces to release contained worm eggs, it gives entire satisfaction. The old shaking machine worked with the bottle corked and was odorless in operation. The new machine works with the bottle uncorked, and does release unpleasant gases, but we have carried a rubber tube from the suction pipe in the laboratory and terminated the tube in a small funnel, shown in the illustration, which is held by rubber bands near and above the mouth of the bottle, and find that the suction serves to carry away practically all of the odors. The bottles used have a capacity of 400 mls, and water is added to the feces to make the total content about 300 mls.

The feces having been thoroughly disintegrated with the mixer, they are next poured through a set of sieves. In my former papers I described a sieve made by soldering brass screening to round tin pans from which the tin bottoms had been cut out except for a flange to which the screen was soldered. In the addenda, I noted that Dr. Cobb, of the United States Department of Agriculture had devised some oblong screens which he had found satisfactory. In making up screens for work here, the round form was abandoned and a screen which is approximately square was made up in the shops. These screens, shown in Fig. 1, are of copper, with inside dimensions about  $7\frac{7}{8}$  inches (20 cm.) square. They are made of two copper strips, swaged together at two diagonal corners, the top half inch of the metal being bent over and doubled back against the side on two opposite sides to form a reinforced rim, and being bent out and back on the other two sides to form a reinforced flange which carries the sieve in a rack. This flange is  $\frac{1}{4}$  inch wide. The copper sides are two inches high. On the bottom of the sieves the metal is bent in to form a flange  $\frac{3}{8}$  inch wide for the attachment of the brass screening, which is soldered to this flange. The screens used have mesh apertures of 6, 8, 10, 14, 16, 20, 60, 100 and 120 to the inch, the latter being about the finest screen that will permit of the passage of the eggs of practically any worm parasite. Each sieve has a number stamped in the front side showing the number of mesh apertures to the inch in its screening. A solid-bottomed copper pan, of the same shape and dimensions as the screens, completes this part of the equipment.

In previous publications, the writer has advocated the use of a set of screens which could be "nested," and which were used in this fashion; the feces were poured through the screen when the latter were set one within another in proper order in a glass dish. While still in the service of the United States Bureau of Animal Industry, I abandoned this plan in favor of the use of a rack, similar to the right hand one of the two racks shown in Fig. 1, constructed with two solid board sides and with the boards grooved so that the edges of the circular pans could be slipped into the grooves. The same type of rack serves even better with the present rectangular sieves, and has been improved in the left hand rack shown in Fig. 1, which is a skeleton construction, lighter and less inclined to warp. This latter rack is made of four upright wooden pieces joined by a series of transverse pieces on the sides and back, and

by a top and bottom piece in front. In examining feces for eggs, this large rack, with a carrying capacity of nine sieves and one solid-bottomed pan, is used, the coarsest screen being at the top and the pan at the bottom. When examining feces for worms passed after the administration of anthelmintics, the smaller rack, with an equipment of six of the coarser screens, using the 60 mesh for the bottom screen to guard against the escape of any worms, is used. The racks serve as storage containers for the sieves and pan when these are not in use.

When examining feces for evidences of parasitism in the shape of eggs or larger material, the feces broken up by the mixer are poured through the set of screens in the large rack and the screened feces collected in the pan. The screens are washed by running clear water through them until the pan is perhaps half full. This pan has a capacity of two liters. Then the pan is pulled



Fig. 1.—Apparatus for comminuting and screening feces, and for washing screens.

out and the contents decanted, sedimented, washed and centrifuged to suit the convenience and desires of the operator. The end result is a slide on which there is a minimum of foreign matter, and this foreign matter and the eggs alike are washed clean and stand out for just what they are in the clean water from which soluble matter and fine suspended matter have been removed. Such a slide can be examined with a maximum certainty of detecting eggs present and with a minimum amount of eye strain.

After the pan is taken from the rack, the remaining sieves are washed by running water through from the top until the water from the last sieve comes away clean; in other words until all the soluble coloring matter and matter fine enough to pass the screens are removed. Then the sieves are examined one at a time in a glass tray of clean water. Parasitic and nonparasitic matter are

now washed clean and appear in their true shapes and colors. There is little likelihood of mistaking vegetable or animal material for worms, and little likelihood of overlooking worms. Substantially the same thing is accomplished in examining feces with the smaller rack and fewer screens to detect worms passed after anthelmintics.

It is necessary that a screen equipment of this sort be kept clean and free from contamination that would give misleading results. To cut down the work of cleaning these screens, the apparatus shown at the left of Fig. 1 was constructed. This is essentially a copper box,  $3\frac{1}{2}$  inches wide, 9 inches high, and



Fig. 2.—Exterior view of dark cell for microscopic work.

10 inches long; just large enough to wash one screen at a time. Water is sprayed in through the fan shaped nozzle at the back, the aperture being cut down by solder plugs at intervals to increase the force of the water. On the front of this box are three overflow lips at the top and a small tube at the bottom; light material floats off through the upper lips, and heavy material, such as sand, is washed out at the bottom. The box is provided with a lid to prevent splashing and is set in a tray to drain any overflow to the sink. In practice, sieves are first rinsed under the overflow and then put in the box. One sieve is washed while the next is being examined. By using very hot water, the sieves are not only

cleansed, but practically sterilized. The mixer is very easily cleaned by holding a damp cloth against the shaft or disk while these are spinning.

So far as sterilization is concerned, however, it is advisable to have formaldehyde, a coal-tar disinfectant, or some other adequate disinfectant, added to the original fecal sample whenever feasible. This is valuable for sterilization and also aids in abating the odor. Where plenty of water is used, the odor is a matter of small moment, as the substances responsible for it are soon washed away.

The writer has long been in sympathy with Dr. Cobb's idea that in doing

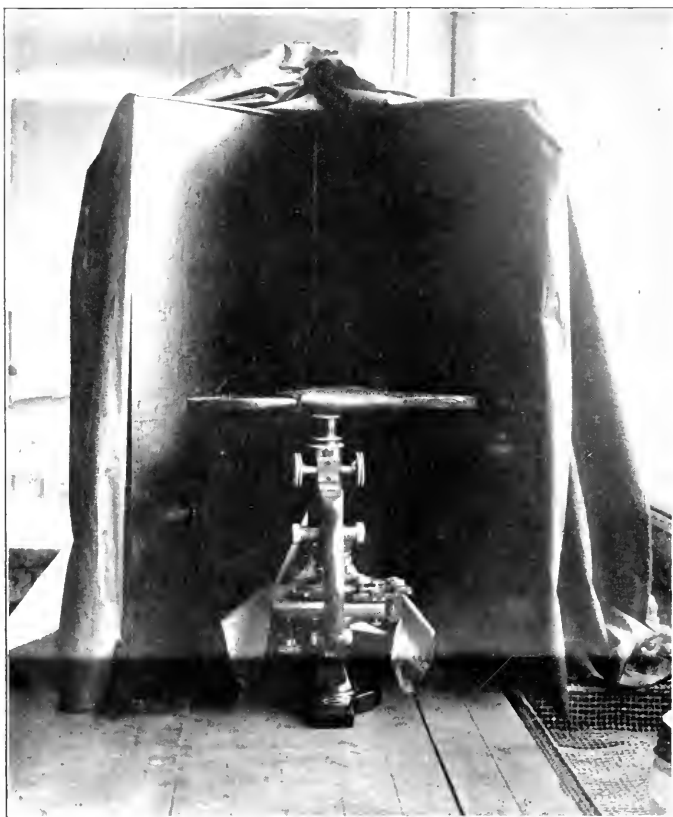


Fig. 3.—Interior view of dark cell for microscopic work.

microscopic work it was advisable to illuminate only the object under the microscope and to cut out, as far as possible, the illumination of the microscope and other extraneous objects in the room. However, the equipment which Dr. Cobb has used to attain his object is rather expensive, the cost of equipping each window with the very satisfactory apparatus which he has devised being about \$80.00 to \$100.00 two years ago, and probably more now. A similar result may be attained by the use of the simple and inexpensive device which is shown in Figs. 2 and 3. This device consists of two boards, each 2 feet long and 1 foot wide, joined at a right angle along the long edges by screws. As shown in Fig.

2, the boards are sawed out at the bottom of this right-angled union to a height of 8 inches, and a reinforcing board fastened along the bottom of the large boards and across the bottom of this aperture to prevent the structure from falling. To further prevent falling, the angle formed by these reinforcing boards is held under a bent nail. A strip of black cloth (lining) is nailed to the edges of the aperture and carried back to the stage of the microscope, permitting light to fall on the mirror. A large piece of black cloth (lining) is nailed to the free edges and top of the large boards, and when this is dropped over the microscopist, it forms a dark chamber which is very restful to the eyes in examining fecal samples for parasite eggs. Midway between the top and bottom of the boards is a triangular shelf, about a foot long on its free edge, and with the free edge hollowed out, beveled and padded to make a head rest. The woodwork of the entire interior of the dark cell is painted black. With this very simple and cheap device, one can examine fecal samples for hours and feel very little of the neck and eye strain that would result from the same amount of work in the open and with the head unsupported. There is only a certain amount of nervous energy available for work; and if this energy is partly dissipated in unprofitable strain on the neck and eyes and in the distraction incidental to seeing the objects and action in the room, there is that much less available for concentration on the work in hand. The use of this apparatus gives a distinct sense of concentration and a feeling of greater satisfaction in what otherwise quickly becomes rather tedious work. As Dr. Cobb has pointed out in his papers, few microscopists seem to appreciate the value of providing suitable and comfortable conditions under which to work, but this does not detract from that value.

When the cloth at the aperture for illumination of the microscope mirror is pushed down and the cloth at the top and back of the dark chamber dropped, the microscope is covered and protected. This arrangement gives adequate protection to the instrument and is handier than the use of a microscope case, as the microscope is always in place ready for use.

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## EDITORIALS

### *Complement Fixation in Tuberculosis*

LABORATORY methods for the early diagnosis of pulmonary tuberculosis have been discussed in this journal, and especially that of complement fixation.<sup>1</sup>

The recently issued Transactions of the Twelfth Annual Meeting of the National Association for the Study and Prevention of Tuberculosis contain articles on this subject from various American laboratories. (Corper, Petroff, Meyer, Bronfenbrenner.)

Many workers in different countries have been busy endeavoring to find an antigen suitable and reliable for this test. As Craig relates, it was the study of Wassermann and Bruck upon complement fixation in tuberculosis that led to the possibility of working out such a test in the diagnosis of syphilis; yet up to the present time, no method of complement fixation in the diagnosis of tuberculosis has been generally accepted.<sup>2</sup>

Corper in a brief review of the literature states that Widal and Lesourd in 1901 were the first to demonstrate deviation of complement in tuberculosis. Bordet and Gengou on whose early observations these serological tests are based

demonstrated in 1903 tuberculosis antibody in the sera of tuberculous animals.

The great difficulty in the practical application of this test has been the inability to discover and to agree upon a suitable antigen.

In the above mentioned series of papers each worker has apparently developed his own type of antigen.

Petroff thinks there may be more than one antibody present in the blood stream, one antibody may react to a protein antigen while a second antibody will react to a lipid antigen. For this reason Petroff devised different antigens, lipid, and protein.

Dielman<sup>3</sup> and Mûch<sup>4</sup> have advocated the use of partial antigens.

Bronfenbrenner and Schlessinger found different samples of Besredka's antigen, although identical in preparation, differed in the amount of lipoids they contained. These workers state that it is necessary to free all antigen from lipoids by separating the protein fraction by precipitation before employing it as antigen.

The early experiments with old tuberculin or even with tubercle emulsion as antigen gave very uncertain results.

Calmette and Massol,<sup>5</sup> in 1912, devised a water and peptone soluble antigen which gave most reliable results, 92.5% of known tuberculous sera yielding positive tests.

Many investigators have followed Besredka's methods, using as antigen a filtrate of an egg meat broth medium on which tubercle bacilli have grown for several weeks.

This antigen, however, has been shown to yield complete binding in a somewhat large percentage of syphilitic sera, a fault of manifest objection to its usage.

Some investigators such as McIntosh, Fildes, and Radcliffe conclude that the antigen must contain living bacilli.

Zinsser and Miller have developed an antigen prepared by triturating living or dead bacilli with dry crystals of sodium chloride then adding distilled water up to isotonicity. This idea would seem to be founded on earlier observations by Gay.<sup>6</sup>

With this antigen the claim is made that cross fixation with luetic sera is eliminated, and that in a large number of cases of tuberculosis the results have been accurate in over 90% of the sera studied. Also by the tests made with this antigen the degree of activity of the disease may be measured.

Such claims may prove of far reaching importance both for the diagnosis and for the prognosis of tuberculosis. E. R. Baldwin,<sup>7</sup> however, is not yet prepared to accept these tests as of value in the diagnosis of tuberculosis although conceding their value for confirmation. He regards their results regarding prognosis as corroborative of the findings of clinicians, in that patients who have apparently arrested pulmonary tuberculosis should not consider themselves out of danger for several years to come; the complement fixation test being usually positive many months after apparent cure, and only gradually returning to negative.

We have employed several antigens in a series of several hundred cases

of pulmonary tuberculosis including early and late types. Those of Calmette and Massol, and of Zinsser and Miller have appeared reliable.

These cases have all been carefully controlled by clinical examinations and by x-ray plates. The results so far would appear to support the claims of the originators of these antigens; that the complement fixation test in pulmonary tuberculosis at least is most satisfactory both for diagnosis and for prognosis. We have rarely seen cross fixation with luetic sera and we feel that in this test we have a serological examination as valuable in pulmonary tuberculosis as the Wassermann reaction in syphilis. Just as in this disease the serum is set up to several antigens, so it would seem best not to rely on only one antigen in tubercle fixation.

The cutaneous tuberculin reactions are, as we know, still positive—denoting a hypersusceptibility—when tuberculous foci are healed, whereas we find the complement fixation test to be negative under these conditions.

Only a vast number of cases can finally settle the value of this reaction. The specificity of the reaction with the recently introduced antigens would seem to be settled.

The varying methods of preparing these antigens, by autolysis, by salt extraction, and by Petroff's method may, in time, be shown to have arrived at a common end in the production of a reliable antigen.

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—G. B. W.

### *Hunger and Appetite*

**A** RATIONAL guidance of the desire for food and means for stimulating or controlling the appetite in pathological states can be obtained only when a knowledge of the determining factors of hunger are known.

Professor A. J. Carlson, of the University of Chicago, has been very fortunate in having in his service a young man, who on account of a complete stricture in his esophagus, has a permanent gastric fistula. The fistula is large enough to allow direct observation of changes taking place in the stomach, and to permit the insertion of various instruments. Prompted, no doubt, by the opportunity which such a subject offers for the study of gastric function, Dr. Carlson and his pupils have conducted an extensive series of researches upon the cause and control of hunger and appetite, and have extended these investigations to include observations upon normal and pathological individuals and upon animals under various experimental conditions. The results of these re-



searches have been recently summarized by Dr. Carlson in a book entitled "*The Control of Hunger in Health and Disease*."\* Any clinician who is interested in gastric disease should not fail to read the volume.

Carlson shows that the normal empty stomach is continually undergoing changes in muscle tone; and, superimposed upon these small contractions, there occur periodically strong contractions of the gastric musculature, which other observers have shown to be simultaneous with the sensation of hunger. These contractions the author calls "hunger contractions," and the period during which they occur, "the hunger period." A high degree of tone in the gastric muscle may be accompanied by a dull sensation of hunger, but it is only during periods when the contractions are strong that the actual pains of hunger occur. The contractions at the beginning of the hunger period are feeble and of slow rhythm, but gradually become more and more rapid and intense, with a corresponding increase in the hunger sensation.

Because Carlson has experienced the sensation of hunger during the contraction of the stomach which follows a sudden distention of its walls, he concludes that the contraction in some way initiates the afferent nerve impulse which gives rise to hunger. He was unable to produce hunger by any form of stimulation of the gastric mucosa, the esophagus, or the intestines.

The author found that during the hunger period there is a great increase in the reflex excitability. Perhaps this may explain the old saying, "cross and hungry as a bear," and also the cause of some of the sensory phenomena which are experienced by many people during hunger. During the hunger period the pulse rate and blood pressure are usually increased. This change may explain the relief of some headache following the taking of food.

Dr. Carlson was unable to produce the sensation of pain by any form of stimulation of the gastric mucosa, short of actual destruction of the cells and nerve endings. He suggests that pain produced in this way, or that occurring in some types of gastritis where even water or gastric juice cause pain, may be due to over stimulation of nerve fibers other than those of "protopathic" pain. Whether such pain is, as Hertz believes, due to hyperperistalsis, or to the actual injury to the gastric mucosa cannot be determined from the present evidence. The only physiological pain from the stomach evidently is hunger pain. Any other pain indicates a pathological process, or the stimulation of hyperexcitable stomach nerves. All attempts to demonstrate any tactile receptors in the mucosa met with failure, but protopathic temperature sense was found to be present.

Some people complain of nausea when they are hungry, and of being hungry when they are nauseated. Dr. Carlson believes that the sensations are not like. Hunger involves the kinesthetic nerves of the stomach, while nausea of gastric origin is due to stimulation of the nerves of the mucosa. He thinks, however, that under certain conditions, as in persons with hyperexcitable vagi, strong hunger may actually pass into nausea.

The feeling of satiety following a full meal appears to involve several fac-

\*Carlson, Autox J.: *The Control of Hunger in Health and Disease*, University of Chicago Press, Chicago, 1916.

tors, none of which depend upon the nerves of the gastric mucosa. The sensation of fullness, accompanied by the memory of taste and smell of food are all required to give this feeling.

The author considers appetite and hunger entirely different sensations. Hunger is produced by the contraction of the muscle of the stomach and the resulting stimulation of receptors in the muscle coats. Appetite, he believes to be primarily dependent upon past experiences with food, a pleasant memory of which is essential. Apparently appetite is a special complex case of the general desire for pleasure, which is modified somewhat by a gastric element. This latter element he demonstrates by placing chemical substances too dilute to cause pain or discomfort, such as alcohol, mustard, pepper, etc., into the stomach. He finds that these affect the consciousness in an opposite sense to pain, and in a way possibly identified with appetite. If such substances are introduced into the stomach without stimulating the taste or olfactory nerves, a sensation akin to the "coming appetite" experienced by normal individuals when eating is experienced.

Such substances introduced during a hunger period abolishes the sense of hunger and gives rise to a pleasant feeling accompanied by thoughts of the dinner table. It is a very significant fact that normal gastric juice, having full acid strength, is capable of giving this sensation. Gastric juice weaker than this abolishes the hunger contractions, but fails to excite what we may provisionally call the gastric element of appetite.

Carlson concludes that the process of feeding is an inherited instinct; a reflex which becomes augmented by the hunger state. In support of this conclusion he finds evidence in the fact that the hungry babe puts everything to its mouth. Experience soon teaches it that food removes the pains of hunger, and gives it pleasure. It, therefore, associates the food with the hunger pains, and its appetite with the pleasure experienced in eating.

Of interest especially to pediatricians are the experiments conducted by Carlson upon the hunger mechanism in babes. These showed that the infant's stomach, immediately after eating, is relatively quiet, but as the stomach empties, feeble tonus contractions appear which increase in rate and activity until, two or three hours after nursing, they end in typical hunger contractions. If food is at hand, infants eat when the hunger sensation is strong enough to make them uncomfortable and usually thrive upon this procedure. If the presence of hunger contractions is biological evidence that the stomach is ready to receive food, the editor wonders if the long pauses between feeding insisted upon by one school of pediatricians is not a rather doubtful means of improving nature.

The author found that the contractions of hunger are much stronger in the young stomach than in the old. That this is not due to the decrease in the metabolism is shown by the fact that even during starvation, when the hunger contractions are augmented, the old stomach never shows the same strength of contractions that the young one does.

The author and one of his pupils investigated on themselves the effect of a fast of five days' duration upon the hunger mechanism. During the fast in both cases there was no decrease in the gastric tonus or in the hunger contractions.

The sensation of hunger was weaker at the end of the period than one would expect from the intensity of the hunger contractions. The appetite increased somewhat on the first days of the fast, but on the last two days it decreased. This weakening of the hunger sensation and the appetite may be due to a depressed cerebral activity at this time. Upon breaking the fast, all the mental depression and much of the weakness disappeared at once. The second day after the fast Dr. Carlson states that he felt as if he had returned from a month's vacation in the mountains, and was able to do an unusually large amount of work. This observation is in keeping with McCullom's statement that periods of increased growth and improved body metabolism follow a fast in animals. The author remarks that the discomforts of starvation were greatest the first two days, but at no time did they amount to actual suffering. He also says that voluntary starvation cannot be considered a heroic act, and that starvation experiments on animals in the interest of science cannot be called cruelty.

Numerous experiments were made to determine the relationship of the nervous system to hunger. These showed that hunger contractions occur in decerebrate animals and during deep sleep, and that they are not under the control of the will. If the hungry individual's attention is directed to other things, the lesser contractions do not affect consciousness, but the more intense ones do, save during deep sleep and at times of exceptional mental activity. Habits of eating do not modify the occurrence of these contractions. Hunger is felt when the stomach is empty, and the body requires food. The sight of food increases the appetite, but does not increase the strength of the contractions in a starving stomach. Carlson found that stimuli received through the gustatory, olfactory, or gastric mucosa nerves, in no case augmented the hunger contractions, but when these stimuli were effectual at all, inhibition resulted, the inhibition, on the whole, being proportional to the strength of the stimulus.

The stomach, completely isolated from its nerve supply, still exhibits hunger contractions although they are modified in strength and rhythm. If the splanchnics alone are severed, psychic stimulation, which usually causes inhibition, no longer has as marked an effect as normally. The cutting of the vagi leaves the stomach in a hypotonic state, but the hunger contractions still persist with alterations in rate, rhythm, and strength. Some intestinal states inhibit the contractions, a fact which may give an explanation of the absence of hunger in many cases of appendicitis, gall stones, etc. The paths by which the augmentary and inhibitory stimuli which affect the hunger contractions reach the brain or the connections which they make in the cerebral cortex with the motor nerves of the stomach have not been determined. Dr. Carlson points out that such work must be accomplished by the clinician, since means for such investigation are not within reach of the physiologist.

Carlson investigated the hunger contractions in conditions known to increase the hunger sensations—such as pancreatic diabetes, phlorhizin diabetes, the hunger following profound hemorrhage and prolonged starvation. In all these conditions he discovered hunger contractions of great intensity. The blood from dogs suffering from the conditions injected into normal dogs during a period of hunger contraction augmented the existing contractions, but

if injected during a nonhunger period, no effect could be demonstrated. The blood apparently contains a hormone under these conditions, which stimulates contractions when the inhibitory factors are weak. The relationship which such a hormone plays is not clear, for, as Carlson points out, it is difficult to account for the periodicity and the abrupt termination which characterize the hunger contractions during a fast on the basis of a hunger hormone secreted by the tissues.

The results of Carlson's researches lead one to believe that the hunger contractions arise automatically within the neuromuscular apparatus of the stomach. The exact stimulus which initiates them is questionable; perhaps a hormone secreted by the tissues or the lack of tension on the walls of the stomach play a role. The movements are modified by the extrinsic gastric nerves in a way analogous to the control which the cardiac nerves have upon the heart.

Alterations in the hunger sensation are known to occur in many pathological conditions. Carlson found that the polyphagia of pancreatic diabetes was accompanied by abnormally strong hunger contractions. Dogs suffering with the mange have big appetites, suffer from the cold, and show very intense hunger contractions. Gastritis, induced in dogs by the administration of large doses of alcohol, caused a suspension of hunger contractions for a day or so. The gastric contractions as well as the desire for food returned, however, before the dogs were able to retain food without vomiting. In these cases hunger and nausea were both present. Distemper, pneumonia, and peritonitis all caused an inhibition of the gastric tonus. Artificial pyloric constriction in the dog caused a great increase in the contractions, and a hyperperistalsis of the stomach. These phenomena are similar to those observed in man with pyloric obstruction.

Patients suffering with diabetes, gastric cancer, and neurasthenia referred to the stomach, all showed abnormally intense gastric contractions. The pains of gastric ulcer apparently arise from hunger contractions, not abnormally increased, but perceived as more painful than hunger pains because of the hyperexcitability of the nerve fibers in the stomach. The gastric pain occurring in gall bladder disease some time after a meal, is probably due to rhythmical contractions of the gall bladder parallel with the hunger contractions of the stomach. In high fevers the hunger mechanism is inhibited.

—R. G. P.

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### *Arteriosclerosis*

CERTAINLY a large proportion of the vascular lesions one sees, especially those in the aorta, is of infectious origin, judging from the microscopic appearances. That is to say, they are typically inflammatory lesions which originate in the media about the vasa vasorum. There is, however, a large number of cases in which these inflammatory lesions are absent, but in which there are evidences of degenerative changes which are represented by fatty plaques and streaks showing through the intima, and others in which the lesions are merely productive ones affecting the intima itself. Practically all investigators who

have endeavored to produce vascular lesions, experimentally, with bacteria, have succeeded in causing inflammatory lesions associated with, in many cases, degenerative changes. Those who have used adrenalin have produced only degenerative lesions.

Bailey<sup>1</sup> has experimented with a toxin—that of the diphtheria bacillus—which he has used in sublethal doses over different periods of time, and studied the effects upon the vascular system and the kidneys of rabbits. In order to discover the relation of high blood pressure, he has used pituitrin in combination with the toxin in one series. Pituitrin was used because it produces no vascular lesions. He has been able to produce, with large doses of toxin, a vascular degeneration involving the entire aorta, the carotids to the base of the skull, the subclavians and iliacs, and, for a varying distance distally, the brachials, femorals, and large abdominal vessels. In combination with pituitrin extensive calcification occurred, due, Bailey believes, to the production of more extreme fatty degeneration. In the kidneys the toxin produced a pronounced vascular and parenchymatous degeneration.

Bailey remarks that the experiments do not show the effects of frequently repeated small doses, and it strikes one that this is what should be shown if the application is to be valuable. An individual does not, during disease, get a large dose of toxin at 10 A. M., and then none for another day or more. He is poisoned by more or less *continuous* absorption of toxic materials which are produced in gradually increasing amounts and then in gradually decreasing amounts. In diphtheria, the course of the disease is short and the period of absorption is brief; in typhoid, the course is longer; in intestinal stasis, it may be very protracted—but in all it is continuous.

The thing that Bailey's work is useful to demonstrate is that a certain type (degenerative) of arteriosclerosis is not microbic—not infectious—in origin, but toxic, and that the lesions may be produced by concentrated materials.

<sup>1</sup>Bailey: Jour. Exper. Med., 1917, xxv, 109.

—P. G. W.

### Thymus

A VERY considerable amount of experimental work has been done upon the thymus, the object of it being to discover whether or not this organ has any important place in the economy of the organism. A large part of this work has been carried out with guinea pigs—the normal laboratory animal. The results have been contradictory. For instance, since 1845 when Restelli performed his first experiments, major reports have been made by more than twenty-five investigators on at least fourteen species of animals. Some have been able to see no results of removal and have inferred that the thymus has either no function in post-fetal life or that none can be demonstrated. Others have seen changes of a transitory character develop and consequently have thought that the thymus exercises a transitory function corresponding to its

own life history. Still others have determined that removal of the thymus is followed by symptoms and pathological changes of a profound character, culminating in death, and have drawn the conclusion that the thymus is essential to life.

A recent communication by Park<sup>1</sup> has to do with a new series of experiments in which the guinea pig was used. In this communication one very striking observation was made which upsets, to say the least, a large amount of previous work. Park found that accessory lobes of thymus derived from the third thyro-pharyngeal pouch, occurring in close association with the parathyroid from the third pouch, were present in the cervical tissue of 11 out of 14 guinea pigs. He believes that the same conditions would have been found in all 14 but for technical errors. In some animals other additional lobes of thymus tissue were found at some distance from the main lobe. The almost impossibility of complete thymectomy without disturbance of other cervical organs is made obvious by these observations, and the previous extirpation experiments of former workers must be discarded.

In Park's animals in which a complete extirpation seemed to have been accomplished, no physiologic effects were observed.

Park recalls that Basch is authority for the statement that herbivora are poor subjects for this sort of experiment, because, very early in these animals, bone has a high calcium content, which acts in a protective manner against the influences that result from cessation of thymus function. And yet it is well known that rickets occurs spontaneously in herbivora, and numerous investigators have reported changes in the skeleton of the rabbit following thymectomy, and one (Klose) has obtained rachitic-like changes in goats. No one, however, has succeeded in producing alterations in the skeleton of the guinea pig by thymectomy. If, as Park says, Basch's observation is true, that removal of the thymus is less apt to be followed by bone changes in herbivora than in omnivora or carnivora, another explanation is that extirpation of the thymus in the former is almost invariably incomplete.

All this recalls the lack of agreement concerning the cause of "thymus death." If Park's observations are true, and especially if they are extended to other groups of animals, then the mechanical cause in these cases must be conceded to be the essential one.

<sup>1</sup>Park: Jour. Exper. Med., 1917, xxv, 129.

—P. G. W.

### *The So-called Mùch-Weiss Tubercle Granules*

MARMOREK, in 1900, noticed that young tubercle bacilli were often not acid-fast. E. Klebs,<sup>1</sup> a few years later, according to Ravenel, published confirmatory and more extensive observations.

In 1907 Mùch,<sup>2</sup> by means of a modified Gram's stain, demonstrated two non-acid-fast forms of tubercle bacilli. Almost simultaneously Michaelides<sup>3</sup> published somewhat similar findings.

Meador,<sup>4</sup> in a very thorough review of the literature concerning tubercle bacilli not stainable by Ziehl, discusses the clinical significance of what are now known as the Mûch-Weiss granules and granular organisms. It is assumed that these may be degenerate types of tubercle bacilli and are non-acid-fast because of the absence of the wax coating.

Fishberg,<sup>5</sup> on the evidence and arguments presented by different investigators, accepts the probability that a diagnosis of pulmonary tuberculosis can be made on the findings of these granules when acid-fast bacilli cannot be discovered in sputum.

There has always been some difficulty in easily demonstrating tubercle bacilli in the pus from cold abscesses, in the discharges from tuberculous ears, in the contents of many tuberculous lymph nodes, and in the drainage from sinuses such as rectal fistulae. It is claimed that in such conditions the Mûch-Weiss granules are especially liable to be discovered.

Some experimentations on guinea pigs would appear to have been carried out to prove that these granules can produce tuberculosis following their inoculation. It must be borne in mind, however, that very few genuine virulent bacilli (less than ten) can infect a guinea pig when injected subcutaneously and that these few bacilli may easily have been overlooked in smears from such discharges.

With all the favorable arguments of different workers, enough animal inoculations have not been carried out and this corroborative evidence is especially lacking in a majority of the reports presented. Neither would it appear that sufficient evidence has been presented from postmortem examinations.

The report of Fraenkel and Mûch<sup>6</sup> that identical granule forms are to be found in the glands of Hodgkins' disease and of lymphatic leukemia structures, would hardly seem to strengthen the theory that these granules stained in this manner are really tubercle bacilli.

In our own investigations we have so far failed to infect guinea pigs with the sputum in which the Mûch-Weiss granules have alone been demonstrated. On the other hand, with large amounts of sputa repeatedly found negative to the Ziehl staining type of tubercle bacillus and in which these Gram positive granules were never discovered, we were able after several trials to infect a guinea pig and the true nature of the disease was determined.

Postmortem evidence is of even greater value. A man of 60, with persistent fever for several years, was pronounced a case of pulmonary tuberculosis by several eminent clinicians. All clinical signs were certainly typical of this disease. Very many examinations of the sputa failed to reveal Ziehl staining tubercle bacilli. The Mûch granules were repeatedly demonstrated by the Weiss double stain. Guinea pig inoculations were negative. The case came to autopsy. Not a trace of tubercle infection could be anywhere detected. The case was one of chronic interstitial pneumonia.

Until more convincing evidence is reported of corroboration by guinea pig inoculation and by postmortem proof, it could hardly seem justifiable to render a diagnosis of pulmonary tuberculosis by the finding of these Mûch-Weiss granules in the sputum of suspected cases.

What the exact nature of these granules may prove to be, it is impossible to surmise. It is to be remembered in such investigations that, if possible, two or three ounces of sputum are to be concentrated and treated and finally reduced to a very small centrifugated sediment. The method is laborious and very time consuming and cannot at this time be recommended.

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<sup>4</sup>Meador: Am. Jour. Med. Sc., Dec., 1915, cl, No. 6, 858.

<sup>5</sup>Fishberg: Pulmonary Tuberculosis, 1916.

<sup>6</sup>Fraenkel and Müch: München. med. Wchnschr., 1910, lvii, 685.

—G. B. W.



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## ORIGINAL ARTICLES

### SPIROCHÆTES\*

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TODAY a spirally shaped microorganism may be called either Spirochæta, Spirillum, Treponema, Spirochaeta, Cristispira, or Saprospira, according to the characteristics of the organism. The choice of the generic name for a given variety is still very much dependent upon the individual views held by different investigators, and this has led to a somewhat chaotic state of affairs in the nomenclature of this group of organisms. This is found to be the case more especially in the medical literature where these minute spiral organisms play an important part as causative agents of certain diseases. Nevertheless, thus far but little attention has been paid to the systematic position occupied by them. Since Ehrenberg,<sup>1</sup> in 1838 introduced a new generic term "Spirochæta" to designate a free living spiral organism which he found in a swamp near Berlin, it remained practically unnoticed until 1904 when Schaudinn<sup>2</sup> stated as his view that so-called Spirochæta constitutes a phase of the life cycle of trypanosomes; hence, that they are of protozoan origin instead of being plants. It may here be mentioned that Ehrenberg, Migula, and other systematists classified the Spirochete under Bacteria, which classification was accepted for nearly seventy years. Indeed, it was not uncommon among medical authorities to employ the terms Spirochæta and Spirillum interchangeably. Medical men may consider the causative agent of relapsing fever as being a Spirillum or a Spirochæta, according to their inclination. This sort of indiscriminate use of terms has gradually extended to other spiral organisms, such as the causative agent of syphilis. According to the old school it was of very little importance whether a spiral organism had one or two polar flagella or a tuft of flagella, so long as both Spirochæta and Spirillum belonged to the same family. On the other hand, Schaudinn and his school maintained that the difference between Spirochæta and Spirillum is no longer so

\*From the Rockefeller Institute for Medical Research.  
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easily disposed of since one is of plant and the other of animal origin. The revolutionary view of Schaudinn was based chiefly upon his observations on a protozoon. *Leucocytozoon ziemanni* is regarded by Schaudinn as a trypanosome found in the blood of the little owl (*Athene noctua*), which is said to undergo a spirochætal stage while passing through an intermediary host. While the accuracy of Schaudinn's observations has been questioned by later investigators,<sup>3, 4, 5</sup> the great impetus which his theory occasioned has had a far-reaching effect upon the development of our present knowledge concerning the organisms generally known as "spirochætes." It was soon after announcing his views that Schaudinn made his famous discovery of the occurrence of *Spirochæta pallida* in syphilis. In their first publication Schaudinn and Hoffmann<sup>6</sup> gave the name "*Spirochæta pallida*" to the spiral organism found in syphilitic lesions because of its resemblance to Spirochætes in general, but within a year Schaudinn recognized certain features (preformed cylindrical spiral filament, difficulty in staining, regularity of curves, etc.), which he considered distinctive enough to classify it apart from the usual Spirochætes (changeable curves, taking on of a violet component of Giemsa, no preformed spiral, ribbon form, etc.).<sup>7, 8</sup> Thereupon he replaced the generic name Spirochæta with a new term Treponema.<sup>9</sup> This all occurred in 1905. But before Schaudinn had had time to decide upon a new generic name for his organism, Vuillemin<sup>10</sup> (1905) proposed that it be called "Spironema." In the meanwhile some authors, particularly in France, commenced to use the term "Spirille." There were also some newer generic names created by still later systematists, such as Microspironema<sup>11</sup> (Stiles and Pfender\*), Borrelia<sup>12</sup> (Swellengrebel), Spiroschaudinina<sup>13</sup> (Sambon), and Spirosoma<sup>14</sup> (Schilling), but these are of no importance today. The only difficulty in choosing the generic name for "*Spirochæta pallida*," lies in the fact that although Schaudinn corrected his error within several months after his discovery, another suggestion had meanwhile been made to answer the same purpose, and according to the international code of nomenclature, Vuillemin's Spironema would have had to receive preference over Schaudinn's own Treponema, had it not been for the fact that the term Spironema as proposed by Vuillemin is not acceptable to those who maintain, like Schaudinn, that the organism of syphilis belongs to the protozoa, because in 1892 it was used by Klebs as a genus of Flagellate.<sup>15</sup> The same name had also been used by Meek in 1864 for a fossil snail. Of course, "Spironema" may be available for any one who holds that "spirochætes" do not belong to Protozoa.† Thus, Gross<sup>16</sup> (1910) used this term to include various spirochætes allied to the spirochætes of relapsing fevers, syphilis, etc., with the specification that he believed these to be of bacterial nature. It may be mentioned that the term "Spirochæta," as taken up by Schaudinn in 1905 in the sense of protozoan organism, had already been used by Michael Sars in 1856 for an annelid genus. It seems that the creation by Schaudinn of the genus Treponema was perfectly justified, although not all the characteristics attributed by him to this genus are found to be distinctive from those of other "spirochætes." Schau-

\*The statement of some authors (Gross and Gonder) that this antedated Schaudinn is erroneous. Schaudinn published his note on October 19, and Stiles and Pfender on December 2 of the same year.

†The use of two identical terms, one in the animal and the other in the plant kingdom, has been known to occur and is permissible. For example, "Bacillus" and "Coccus" are found in zoological as well as in botanical genera.

dinn did not live long enough to witness the gradual modification which the Spirochæta question went through. As a result of the works of various systematists and zoologists, we are brought to realize that the original *S. plicatilis* described by Ehrenberg in 1838 is an entirely distinct organism and bears little relation to the other organisms which we now call "spirochætes." We also know that the latter should no longer be designated as spirochætes, and that the spiral organisms found in the crystalline style of various mussels are neither trypanosomes, as held by Perrin,<sup>17</sup> nor typical spirochætes, but form another group which may be seen to possess one or more genera. These facts were revealed after the death of Schaudinn by the careful studies of Novy and Knapp,<sup>18</sup> Schellack,<sup>19</sup> Gross,<sup>20, 21</sup> Zuelzer,<sup>22</sup> Gonder,<sup>23</sup> Dobell,<sup>24, 25, 26</sup> Hoelling,<sup>27, 28</sup> Fantham,<sup>29, 30</sup> Swellengrebel,<sup>31</sup> Bosanquet,<sup>32, 33</sup> and others. Although much light has been thrown upon the structure of these organisms, no definite conclusion has yet been reached as to the affinity of the "spirochætes" in the system of natural history. While there are still some who consider "spirochætes" as allied to bacteria and others who regard them as of a protozoan nature, there now appear to be certain authors who are inclined to set them apart both from bacteria and protozoa, and classify them apart in the domain of the Protista, i. e., belonging to neither plant nor animal. Dobell<sup>24</sup> represents this view, and Doflein<sup>34</sup> compromises by calling them "Proflagellate," and placing them between Bacteria and Protozoa. Zuelzer<sup>22</sup> holds a somewhat similar opinion to that of Dobell. In order to bring up some of the more important data relative to the question of classification, we shall now review the present situation.

As remarked at the beginning of this paper the Spirochæta of Ehrenberg was regarded as a genus of the family of Spirillacea, and no question was raised in regard to its possible affinity with the Protozoa until the publication of Schaudinn's fascinating observations on *Leucocytozoon ziemanni*. Since that time there have appeared numerous partisans of Schaudinn's view that so-called spirochætes are of protozoan nature. Their main contentions are as follows: (1) Longitudinal division as the mode of multiplication, (2) presence of an undulating membrane; (3) high degree of bodily flexibility; (4) absence of cell membrane; (5) absence of a motor organ such as the flagella; (6) presence of a periplastic process; (7) peculiar nuclear arrangement; (8) band-like bodies; (9) encystation or resistant form; (10) a certain periodicity in their pathogenic activity in the infected hosts, and (11) effect of certain chemicals such as sodium taurocholate, saponin, etc., which bring about the dissolution of these spiral organisms and thus offer a contrast to the great resistance shown by bacteria (especially spirillum) to these substances. The foregoing characteristics tended to place the Spirochæta in the Flagellate group, but subsequent studies by different investigators, especially those who have employed a more recent and approved cytological technic seem to indicate that many of the above criteria were based upon erroneous or insufficient observations. According to the observations of Dobell,<sup>24</sup> Gross,<sup>16, 20, 21</sup> Zuelzer,<sup>22</sup> Swellengrebel,<sup>31</sup> Novy and Knapp,<sup>18</sup> and others, the following features are characteristic of "spirochetes."

1. In the case of the majority of "Spirochetes" transverse division is the only mode of multiplication (Koch, Levaditi, Fraenkel, Novy and Knapp, Bor-

rel, Gross, Zuelzer, Swellengrebel, Schellack, etc.). Only in certain pathogenic small varieties has the occurrence of longitudinal division been reported.<sup>7, 35</sup>

2. No undulating membrane has been definitely demonstrated in any Spirochæta. The alleged undulating membrane depicted by Perrin<sup>17</sup> and Schaudinn<sup>36</sup> in the dried preparations of certain mussel spirochætes is an artefact brought about by improper fixation; namely, the torn crista of a cristispira.<sup>20</sup>

3. The alleged chromatin rods and spirals described by Perrin in the case of certain mussel spirochetes known as Spirochæta balbianii (Cristispira of Gross) are now said to be nothing but a distorted arrangement of volutin substance or chromidial granules which under optimum fixation gather themselves along the walls of the chambered structure of the cell body.

4. The absence or presence of cell membrane seems to depend upon the variety of "spirochætes." Thus the original type organism of Ehrenberg was described as being devoid of a membrane and is still so regarded by all who have studied this organism. On the other hand the mussel spirochætes and various small parasitic species are now said to be provided with a thin but elastic membrane which cannot be differentiated from the cell body by means of staining reactions. The presence of a membrane would suggest a close affinity with Spirillum, but the latter has a stiff nonelastic membrane.<sup>22</sup>

5. In regard to the motor organ no generalization can be made. The original type organism of Spirochæta and all mussel "spirochætes" are devoid of any special motor apparatus. On the other hand, a terminal process, consisting of a delicate, elastic filament with minute, regularly set curves, may in the case of various small parasitic spirochetes be found to project from one or both ends of the body. Borrel<sup>37</sup> and Zettnow<sup>38</sup> obtained some preparations in which Spirochætes of fowl spirillosis and relapsing fever appeared to possess peritrichal flagella, but this must have been a case of artefact formation, as no one has since been able to confirm their findings. Schaudinn considered the terminal process to be identical with the periplastic appendage of a flagellate.

6. Certain spirochætes such as *S. balanitidis* and *S. buccalis*, etc., were said by Schaudinn<sup>36</sup> Hoffmann and Prowazek,<sup>39</sup> to have a flattened, ribbon-formed body. Later investigators hold that the body is cylindrical and round on section.

7. Encystment, or the resting stage, such as observed in protozoan organisms, has been suggested<sup>17, 40</sup> as existing, but never satisfactorily proved.

It will be seen that the findings of later investigators deduct much of the foundation upon which the protozoan theory of "spirochætes" had been based. Not only do they separate the spirochætes from the Protozoa, but they also bring out certain new facts which make it difficult to include them among the Bacteria as was formerly done by those who opposed the view of their protozoan nature. As has been briefly remarked, the spiral organisms called spirochætes are not of uniform structure, but according to recent investigations, fall under several great divisions. It was owing to the imperfection of the methods of study that the free living forms and numerous parasitic varieties were at one time all held to belong to the same genus. Since the introduction of darkfield microscopy,<sup>41</sup> many points which could not be satisfactorily determined with stained specimens have been carefully checked up, and the entrance into the field of certain excellent

cytologists has helped to clear up many points relating to the systematic grouping of these organisms. These cytologists made extensive series of comparative studies, at the same time carefully examining the structure of bacteria, spirilla, spirulina, and oscillaria.

As has been pointed out by Bütschli,<sup>42-43</sup> bacteria are composed of a central body and a plasmatic layer. The former contains volutin granules and some chromidial elements. The spirillum has a series of chambers, each of which is constructed like a single bacterial cell. Both are covered with a stiff cell membrane. The structure of Spirulina is similar to that of Spirillum, differing from the latter by the highly flexible character of the membrane. Now, a very similar structure was demonstrated by Gross<sup>20</sup> in the body of mussel spirochætes and speedily confirmed by Dobell,<sup>24</sup> Zuelzer<sup>22</sup> and others. Gross, Dobell, and Zuelzer all agree that the original free-living Spirochæta described by Ehrenberg is a unicellular organism which bears no relation either to the mussel spirochætes or to the small parasitic varieties. This fact implies the dissociation of the long used term Spirochæta from those organisms which in reality were commonly known as "spirochætes." Odd as it may seem, the true Spirochæta has been but rarely studied, even by biologists, and certainly not to any great extent by medical men who have so much to do with the so-called "spirochætes."

Gross<sup>16</sup> was the first person who proposed to distinguish the true Spirochæta from the other varieties of spirochætes by creating new genera for the latter which, according to his studies, could not be classified with Spirochæta in the strict sense of the term. Thus for the latter type he created the name Cristispira (those with Crista), for the large parasitic "spirochætes" in fresh shell fish Saprospira (those without Crista), and the small parasitic varieties, including all pathogenic species, he designated as Spironema. Gross maintains that Cristispira, Saprospira and Spironema belong to the bacteria, and places them under the family name of Spironemacea. Gross<sup>44</sup> and Bosanquet<sup>32</sup> recorded a few instances in which certain mussel "spirochætes" went into spore-formation comparable to the true bacterial feature.

Dobell and Zuelzer both admit the striking resemblance between the chambered structure of Spirillum and Cristispira, but cautiously avoid accepting the bacterial theory of Gross on the ground that the last named organisms have a more elastic and flexible membrane and that they are not necessarily bacteria. Dobell, as has been stated, has proposed a new family name Spirochætoidea which should include not only Gross' Spironemacea but also Spirochæta. Dobell does not accept Gross' Spironema, as it was applied to a flagellate in 1892 (Klebs), but retains Schandinn's Treponema to designate all small parasitic and pathogenic varieties. He does not consider that there is a sufficiently essential difference between them to warrant two genera. Zuelzer regards the affinity between the mussel "spirochætes" and Spirulina (one of the Cyanophycean genera) as being much closer than that between these types and Spirillum. On the other hand Gonder<sup>45</sup> accepts the classification of Gross more completely. He does not, however, share Gross' view that these organisms are definitely of a plant nature, holding that certain features indicate their partial affinity to the protozoa. He also differs from Gross in including Spirochæta under Spironemacea and in retaining

Schaudinn's term *Treponema* for the organisms of syphilis and yaws and such affections, while he accepts Gross' term *Spirosonema* for other varieties such as the "spirochaetes" of relapsing fevers, tick fever, etc. The situation is still confused.

#### CLASSIFICATION AFTER GONDER (1912).

##### SPIRONEMACEA (GROSS, 1910).

<i>Spirochaeta</i> ..... (Ehrenberg, 1838)	Type: <i>Spirochaeta plicatilis</i> , etc., all free living.
<i>Cristispira</i> ..... (Gross, 1910)	Type: <i>Cristispira balbianii</i> and other varieties found in mussels.
<i>Spirosonema</i> ..... (Vuillemin, 1905)	Type: <i>Spirosonema recurrentis</i> , and other parasitic and pathogenic varieties living in blood.
<i>Treponema</i> ..... (Schaudinn, 1905)	Type: <i>Treponema pallidum</i> , <i>Treponema pertenue</i> , and other varieties with closely set spirals.

#### CLASSIFICATION AFTER GROSS (1912).

<i>Spirochaeta</i> ..... (Ehrenberg)	Type: <i>Spirochaeta plicatilis</i> . Unicellular organism without a membrane or flagellum, highly flexible. Free living. Transverse division.
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##### SPIRONEMACEA (GROSS, 1912).

<i>Cristispira</i> ..... (Gross, 1910-11)	Including different varieties living in certain mussels. <i>C. balbianii</i> , <i>C. anodontæ</i> , <i>C. pectinis</i> , etc. All possess a crista. Chambered structure of the body. Sporulation. Transverse division.
<i>Saprospira</i> ..... (Gross, 1911)	Similar to the foregoing except that there is no crista. Found in foraminiferous sand. Sporulation. Transverse division.
<i>Spirosonema</i> ..... (Vuillemin, 1905)	Including small parasitic varieties: <i>S. pallidum</i> , <i>S. pertenue</i> , <i>S. recurrentis</i> , <i>S. gallinarum</i> , etc. Probably multicellular (or chambered). Transverse division. Flagella or terminal thread present.

#### CLASSIFICATION AFTER DOBELL.

##### SPIROCHLETOIDEA (DOBELL) 1910-1911.

<i>Spirochaeta</i> ..... (Ehrenberg, 1838)	Free living forms, fresh water or marine. <i>Spirochaeta plicatilis</i> (Ehrenberg) <i>S. gigantea</i> .
<i>Treponema</i> ..... (Schaudinn, 1905)	Parasitic in animals, vertebrates and invertebrates. <i>T. pallidum</i> (Schaudinn), <i>T. recurrentis</i> , <i>T. dentium</i> , etc.
<i>Cristispira</i> ..... (Gross, 1910)	Parasite in Lamellibranchiata (mussels). <i>C. balbianii</i> certes, <i>C. anodontæ</i> , <i>C. pectinis</i> , <i>C. veneris</i> .

#### CLASSIFICATION AFTER MIGULA (1897).

Bacteria .....	Coccaceæ, Bacteriaceæ, Spirillaceæ, Chlamydobacteriaceæ and Beggiatoaceæ.
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##### SPIRILLACEÆ.

<i>Spirosoma</i> .....	Rigid; no organ of motion.
<i>Microspira</i> .....	Rigid; one seldom two or three, polar wavy flagella.
<i>Spirillum</i> .....	Rigid; polar tufts of 5-20 flagella, mostly semi-circular or wavy.
<i>Spirochaeta</i> .....	Flexuous, motion organ unknown, probably an undulating membrane.

CLASSIFICATION AFTER SWELLENGREBEL.

Bacteria....	{	Spirochætaceæ.....	{	More or less distinct properties of protozoa but not much more so than the bacteria capable of forming S or Fe in contrast to those producing nitrification. Plasmolysable like the Spirilla.
		Spirillaceæ		
		Coccaceæ		

CLASSIFICATION AFTER LEVADITI (1912).

SPIRILLACEÆ (MIGULA).

1.		2.	
Sub-family, Spirillaceæ (Swel- lengrebel). Not flexible.		Sub-family, Spirochætaceæ. Spirally flexible, sur- rounded by periplastic cover, with or without flagellum-like appendage.	
1.	2.	1.	2.
Genus, Spirillum.	Genus, Vibrio.	Genus, Spirochæta. No preformed spiral; loss of spiral form at rest.	Genus, Treponema. Preformed spiral; no visible undulating membrane.
		a	b
		No flagel- lum with undulat- ing mem- brane.	Flagellum, but no undulat- ing mem- brane.
		One or two terminal flagella.	Peritrichal flagella.
		.....	.....
		<i>S. lutealis</i> , <i>S. balantidis</i> , <i>S. refringens</i> .	<i>S. gallinarum</i> , <i>S. duttoni</i> , <i>S. obumetleri</i> (?)
			<i>T. pallidum</i> , <i>T. framboesia</i>
			<i>S. dentium</i> .

Let us consider each of the groups in some detail on the basis of a newer classification.

*Spirochæta*.—According to Schaudinn,<sup>36</sup> the type organism of *Spirochæta* possesses certain features which are also found in trypanosomes—such as an undulating membrane, periplastic fibrillar process, longitudinal division, etc. But this apparent resemblance has been shown to be erroneous. Thus, according to the latest contributions made by Zuelzer,<sup>22</sup> the original type organism, *Spirochæta plicatilis*, has no chambered structure but is provided with a straight fibrillar axial filament surrounded by a plasmatic spiral layer which covers it unequally in different places. The organism consists of a single cell. Volutin granules which can be demonstrated by certain microchemical reactions are regularly disposed within the plasmatic layer. During motion the plasmatic layer

at a given position becomes thickened or reduced in volume according to the current of the substance. The spirals of the plasmatic layer surrounding the straight axial filament occur regularly and closely, while the whole body shows several irregular undulations. There is no flagellum or periplastic terminal process, and no membrane has been demonstrated. It measures 100-200  $\mu$  on an average, sometimes attaining a length of 500  $\mu$ , whereas it is only 0.5-0.75  $\mu$  in width. Unlike the other spiral organisms bearing the name of Spirochæta (undoubtedly indiscriminately applied) the members of this group of real spirochætes do not swim, but their locomotion is effected by a creeping movement along the surface of a supporting object. Multiplication is brought about by transverse division which is effected by a thickening of a certain part of the axial filament where a cross fissure takes place, followed by the strangulation of the plasmatic layer at the corresponding spot. Since Ehrenberg described the first species, four more species have been added, one by Cantacuzène<sup>21</sup> in 1910, and three by Zuelzer<sup>22</sup> in 1912. They are all free-living and are not known to be responsible for any pathological conditions in either human beings or animals.

Since the essential characteristics of the group of true Spirochæta do not agree with those of various other species hitherto unreservedly called spirochætes, the necessity of reclassification became apparent as soon as these facts were known about 1910, whereupon Gross, Dobell, and others undertook special studies in this connection. As has been mentioned, Gross, Dobell, and Gonder all possess their individual ways of classification, but all agree on one point; i. e., that the majority of the organisms known as spirochætes are not spirochætes in the strict systematic sense and must, therefore, be differently designated. Gross was the first to do this, and he was followed by Dobell and Gonder who introduced some modifications, but it seems that the family term Spironemacea of Gross has found a wider acceptance than Dobell's Spirochætoidea, although both include practically the same constituent organisms under a slightly different generic name. Thus Dobell accepts Gross' generic names *Cristispira* and *Saprospira* (provided that this genus can be recognized by other investigators) to cover the varieties found in shell fish, while preferring to use *Treponema* instead of *Spiro-nema* as proposed by Gross. Dobell's family Spirochætoidea comprehends, besides all the constituents of Gross' family Spironemacea, the genus of the true Spirochæta. Whether the segregation of Spirochæta from the other genera composing Spironemacea is justified or not seems still debatable, inasmuch as the differences between the genus *Cristispira* and the genus *Spironema* are, I believe, no less striking than those which induced Gross to separate the Spirochæta from them. According to personal observations on small "Spirochætes" there seem to exist more affinities in the structure of true Spirochæta and the small parasitic varieties than are assumed by Gross and other investigators. For the present I will dwell upon different groups of organisms, which those investigators have classified as separate genera, and in order to give a basis for further development of the subject, I propose to employ the new generic names proposed by Gross, without, at the same time, committing myself to his views.

*Cristispira*.—This genus was created by Gross in 1910 for the large saprophytic commensal spiral organisms found in the alimentary canal of certain varie-



ties of shell fish. They are chiefly found in the crystalline style which is a jelly-like projection in the stomach. The most unique feature of the genus is the presence of a crista or ridge, which extends spirally along the whole length of the body, whence the name *Cristispira*. Certes<sup>47</sup> considered the type organism of the genus *Cristispira balbianii* to be a trypanosome on account of the presence of an undulating membrane (later recognized by Gross as a ridge) and it has been called *Trypanosoma* or *Spirochæta* indifferently. Laveran and Mesnil<sup>48</sup> in 1901 regarded it as allied to the bacteria. Perrin<sup>17</sup> in 1905-1906 took up the subject and arrived at the conclusion that it has many features in common with the trypanosomes. This he observed from stained preparations in which he found an undulating membrane, a spirally arranged nuclear rod, as well as various mitotic figures and longitudinal division. Perrin's observations were in part confirmed by Keysselitz,<sup>49, 50</sup> Swellengrebel,<sup>51</sup> Hoelling,<sup>27, 28</sup> Gonder,<sup>23</sup> and Fantham,<sup>29</sup> but a later investigation of Schellack<sup>19</sup> brought out an entirely different set of facts. According to Schellack the undulating membrane and spiral nuclear rod or alleged karyokinetic figures are an artefact caused by improper fixation (dry method). In properly fixed preparations the cell body is composed of an alveolar protoplasm and contains a number of transverse walls. In his later works Gonder<sup>45</sup> confirmed Schellack's observations. Zuelzer<sup>22</sup> and Dobell<sup>24</sup> found chromatin (and volutin) granules to be deposited along the surface of the transverse septa, while Gross<sup>20</sup> failed to see any chromatic granules in *Cristispira*. On the other hand, Hoelling thinks that the entire cell body is saturated with diffuse chromatin substance. The chambered structure of the cell body is regarded by Gross as a sign of the multicellular nature of the organism, but many authors hesitate to accept this view, maintaining that it is a single organism with numerous cross septa. Gross, Zuelzer, and Dobell all agree that the cell body is surrounded by a strong membrane similar to that found in bacteria, although Zuelzer distinguishes it from the latter by its high flexibility. They found that the membrane had a double contour and protected the cell body from the solvent action of various substances such as saponin as well as from acids and alkalis, a fact explained by Gonder as not necessarily due to the presence of a membrane but to the more concentrated external fibrillar layer on the cell surface. In fact, Gonder described a fibrillar appearance of the external layer of the cell body after the organism had been acted upon for some time by certain chemicals.<sup>45</sup>

Opinions still vary as to the origin of the ridge or crista. Earlier workers viewed it as an undulating membrane.<sup>17</sup> Gross, Zuelzer, and Dobell hold that it is a superposed structure having no direct connection with the cell body, while Schellack regards it as a true periplast traversed by numerous fibrils. He believes that the so-called undulating membrane of the authors of the *Cristispira* is an artefact produced by defective technic. Hoelling, as well as Fantham and Porter, entertains a view similar to that of Schellack, and the presence of a myoneme in the periplast was even maintained by Fantham and Porter. MacKinnon<sup>51</sup> and Vlès<sup>52</sup> were unable to demonstrate any myoneme in the periplast, although Borrel and Cernovodeanu<sup>53</sup> assume that there exists a myoneme in the membrane which enables it to flatten or fold the ridge. When the organism is subjected to macerating or solvent agents (saponin, acid, alkali, etc.) the mem-

brane is first attacked. The delicate fibrils become quite distinct in the course of dissolution, but the whole structure finally disappears completely, showing the plasmatic nature of the membrane. The cell body is much more resistant.

Division is exclusively transverse, according to the investigations of Schellack, Gross, Zuelzer, Dobell, Laveran, and Mesnil, while earlier investigators (Perrin, Keysselsitz, Gonder, etc.) considered it longitudinal. Fantham and Porter,<sup>35</sup> working with *S. obermeieri* and *S. duttoni*, found both modes of division occurring. It is possible that a peculiar mode of division, described by Gross<sup>20, 54</sup> as an *incurvation*, might have been the cause of mistaking it for longitudinal division. Incurvation is a phase of the transverse division of *Cristispira*, whose body first doubles up (incurvates) at the segment where the fission is to take place and then after some time completes the process. During the incurvation both halves of the organism intertwine and simulate a stage of longitudinal division.

Sporulation was described by Gross<sup>44</sup> who saw a *Cristispira* produce a series of somewhat smaller, highly refractile, oval bodies out of the square chambered structure of the cell body. These oval bodies were seen to separate into individuals, but no new *cristispira* could be made to sprout out of these bodies (or so-called spores). Bosanquet<sup>32</sup> made a similar observation. The question of sporulation is still open to further confirmation and is very important in view of the divided opinion regarding the affinity of this group in the system.

The cell body is highly flexible, round on section, wavy or spirally wound, possessing not more than three or four curves. There are neither flagella nor terminal projections, except in one small species, *C. spiculifera*, which Shellack described as having a terminal filament.

There are about 18 known species which inhabit different varieties of shell fish belonging to nearly twelve different genera of Lamellibranchs, including common oysters and fresh water mussels. These genera are *Ostrea*, *Anodonta*, *Chama*, *Pinna*, *Macra*, *Pecten*, *Modiola*, *Lima*, *Gastrochæna*, *Saxicava*, *Tapes*, and *Umo*. *Cristispira balbianii* and *C. anodonta* are the largest species and measure 100-130  $\mu$  in length and 3-5  $\mu$  in width, while the smallest representative of the genus, *C. papillosum*, measures but 18.5-20  $\mu$  by 1.1-1.4  $\mu$ .

*Saprospira*.—Gross<sup>20</sup> proposed to introduce this genus in order to group together a new species of mussel "spirochætes" which distinguished themselves from *Cristispira* by the absence of a crista. Their habitat and other cytological features are the same as those noted in the *Cristispiræ*. According to this investigator, *Saprospira grandis* and *S. nana* undergo multiple transverse division and bear a more distinctly bacterial aspect.

*Spiroinema* and *Treponema*.—Under *Spiroinema*, Gross classified all the pathogenic and small saprophytic varieties. Dobell<sup>24</sup> substituted *Spiroinema* for Schaudinn's *Treponema* on the basis that the former term was applied to a flagellate, *Spiroinema multiciliatum*<sup>15</sup> by Klebs in 1892; for he did not consider it necessary to create two genera out of these organisms. Gonder still hesitates to drop the distinction between the group of "blood spirochætes" and that of "tissue spirochætes," the latter containing *Treponema pallidum* as type organism. While Schaudinn's original criteria for *Treponema* are no longer valid as regards several

points, Gonder proposes to retain the term *Treponema* for the pallidum group and to accept *Spirosonema* for the more irregularly curved, wavy varieties to which most of the "blood spirochætes" and saprophytic parasites belong. From personal observations I believe the differences between the two groups to be differences of degree, not of quality. They should belong to one and the same genus as may be seen from the characteristics enumerated below. *Spirosonema* and *Treponema* have a slender, cylindrical, spirally wound, highly flexible body, which exhibits serpentine, cork-screw-like, and sometimes lashing movements. The spiral curves are partially stretched and drawn together with a certain rhythm, so that an actively motile organism resembles a spiral spring which is alternately drawn out and relaxed. When reduced in motility the organism may rotate along its axis in one and then in another direction without changing its curves. In certain species a lateral bending or swinging motion of one half of the body may be seen. It seems to be the general rule that the more active and energetic an organism is, the less rigid are its curves. On the whole the pallidum group (*Treponema*) exhibits a less energetic motility than the heavier group (*Spirosonema*) which it relinquishes much sooner than the latter. Therefore, it is only in perfectly fresh material (such as that obtained from an experimental syphilitic lesion in animals at the moment of examination) that the stretching of the curves, as in the case of so-called *Spirosonema*, can be recognized. This point can be clearly demonstrated in a section of syphiloma in a rabbit's testicle fixed immediately after removal from the animal. Here we find the organisms showing most striking irregularity of curves very unlike the accustomed picture of regularly curved specimens found in a section obtained from postmortem material, such as a tissue from macerated congenitally syphilitic fetus (Flexner)<sup>55</sup> or from a preparation made after the organism has become sluggish. The reverse is also true. A *spirosonema* from a case of relapsing fever is always wavy and irregularly curved in a stained preparation, but it is much more regular when observed under the dark-field microscope and becomes completely regular when nearing death as a result of being exposed to progressively unfavorable conditions. In a culture where the motility is somewhat less active the organism appears just as regularly curved as a *treponema*. The sudden death of these organisms leaves them in a state of motion, hence their irregular curves.

The body of *Spirosonema* is much heavier than that of *Treponema* and in relation to different dyes it may be stated that the former takes on a more bluish component of Giemsa's solution than the latter, which usually takes on the red. In regard to the structure of the cell body, the minuteness of these organisms precludes the possibility of obtaining much information by means of our present methods of differentiation. Many authors assume the presence of a membrane analogous to the periplast of a flagellate and believe that it can be demonstrated by means of maceration. In one species of *Spirosonema*, Prowazek<sup>10</sup> assumed a central axial filament surrounded by a layer of cytoplasm. The active motility exhibited by these organisms led some investigators to suggest the existence of contractile fibrils or a myoneme in the cell body. My observations on fresh specimens obtained from pure cultures of these organisms support the view that these *spirochætes* are provided with an axial spiral filament covered with a layer of

protoplasm. On the surface of the cell body there is a thin membrane which can be detected when the organism undergoes degeneration. At this stage the cytoplasm becomes so rarefied—i. e., it escapes from the space which it occupied—that the axial filament and the membrane can be easily recognized. In a subsequent phase the membrane also disappears, leaving the axial filament denuded. This is a common phenomenon in the cultivation of this group of organisms. Schellack<sup>19</sup> maintains that the external layer of the cell body stains red with iron hematoxylin eosin, while the inner layer takes on a dark bluish tint, hence the former is of ectoplasmatic and the latter of endoplasmatic origin. Gonder<sup>56</sup> describes an ectoplasmatic layer in *Spironema vesperuginis*. Fantham and Porter<sup>35</sup> as well as Prowazek<sup>40</sup> mention the existence in Spironemata of an undulating membrane, as was originally suggested by Schaudinn<sup>36</sup> owing to a wavy movement which he observed to travel through the body of a resting spironema. Gross and Zuelzer failed to demonstrate any such particular structure. Another important feature of Spironema and Treponema is the presence of a terminal appendage projecting from the end of the cell body. The bodies of Spironemata and Treponemata taper at both extremities from which is sent out a very fine terminal thread, at one or both ends. The length of the terminal appendage may reach  $\frac{1}{3}$  to  $\frac{1}{2}$  of the body and is immeasurably thin. In old cultures, especially when grown in a fluid medium, these terminal appendages are much heavier and more easily recognized than in a specimen derived direct from the natural habitat. The terminal filament is provided throughout its length with numerous, closely set, regular curves.<sup>57</sup> It is rigidly joined at the pointed ends of the body or sometimes in such a loose manner as to permit the joint to bend at any angle to the long axis of the organism.<sup>6</sup> No proper motility can be discerned in the appendage, which is elastic. In certain specimens an active swinging or jerking movement can be seen to be transmitted by the organism, which is able to do this by means of its contractile element (myoneme?) contained within the body. In several instances in which the cultivated *Spironema recurrentis* had been exposed to the solvent action of certain chemicals (saponin, sodium taurocholate, etc.), I have observed many denuded axial filaments (their cytoplasmic layer having been dissolved) to which the terminal filaments were also attached. Suddenly I saw some of the terminal projections commence active jerking and swinging motions. The skeletal axial filaments still remained. By means of careful examination it was found that there was a pair of highly refractile, round bodies attached to the skeletal filaments near both extremities. These bodies, which measured about  $0.5 \mu$  in diameter, appeared to have some contractility as suggested by the alternate change in the degree of the refraction of light. Whether or not these bodies represent some sort of myonemous elements cannot be definitely stated, but it is significant that similar nodules, if not in pairs, can be seen to travel from one point to another in an actively motile spironema. Prowazek<sup>58</sup> once called attention to the phenomenon of plasmatic condensation in the body of *Spironema gallinarum*.

The nature of the terminal appendage is not known. Many authors (Hoffmann, Prowazek, etc., on *S. buccalis* and *S. balanitidis*; Novy and Knapp on *S. recurrentis*) view it as a prolongation of the periplastic fibrils which are in

connection with the periplast. Others regard it simply as a drawn out part of the cytoplasm produced at the line of division. I am inclined to think that the terminal projection with regularly set curves is a separate part not directly connected with the membrane, nor existing as a prolongation of the axial filament. It is connected with the cell extremity by means of a tendinous substance. It resembles the flagellum of certain bacteria, inasmuch as it is similarly elastic, finely set with regular curves, and visible under the darkfield microscope. On the other hand, a great many of the bacterial flagella cannot be demonstrated in a fresh preparation even by means of a darkfield illumination. Zettnow,<sup>38</sup> Borrel<sup>37</sup> and Fraenkel<sup>39</sup> obtained preparations of *S. recurrentis*, *S. gallinarum* and *S. duttoni* in which peritrichal "flagella" were shown by means of flagella staining methods, but these flagella-like fibrils are now regarded as fibrils which have become detached from the external layer of the organisms through maceration. By means of the lucidol method of Szécsi,<sup>60</sup> Gonder<sup>45</sup> succeeded in staining one fine terminal projection at each end of *S. recurrentis* as did also Wolbach by the adoption of Casares Gil's<sup>61</sup> method.

There are several views regarding the mode of multiplication. The theory most generally accepted is that these spirochetes undergo transverse division like bacteria, differing from the latter, however, in not forming a wall at the point of division. The division is effected by means of a thinning out process of the protoplasm which for a time bridges the two newly formed daughter cells. Finally they separate by the severance of the connecting thread. Novy and Knapp<sup>18</sup> described a cleft formation at the point of division. The view of the transverse division is held by Koch, Novy and Knapp, Metchnikoff, C. Fraenkel, Borrel, Laveran, Sobernheim, Gross, Thesing, Schellack, Nakano,<sup>62</sup> and others. On the other hand Schaudinn, Hoffmann, Hartmann, Keysseltz, Herxheimer, Prowazek, Gonder, Fantham, and Porter support the theory of a longitudinal division as in the flagellates. Indeed, Krysztalowicz and Siedlecki<sup>63</sup> in 1905 went so far as to propose the term "Spiroflagellata" under Mastigophora. I have also observed instances in which the phenomena could only be explained by longitudinal division. Thus, in pure cultures of various spirochetes and treponemes we find forms in which a longitudinal cleft can be traced in the somewhat heavier specimens. The cleft may run but a short distance, or one-third, one-half or almost the entire length of the body. In some specimens the cleft widens up and causes one-half of the body to be split into two limbs (two daughter cells in half separation). Observed under the darkfield microscope the process is seen to be slow. It may be added that it is tedious to actually follow up the entire process of any mode of division under the microscope, no matter whether this be transverse or longitudinal. As may easily be conceived, those who hold the theory of transverse division argue that the forms held by their opponents to be a stage of longitudinal division are formed by two entwined spirochetes which having been produced by transverse division are still connected by a delicate plasmatic bridge. This argument, however, can also be used in the reverse sense in favor of longitudinal division, as it is also possible that the two daughter cells which have just undergone cell division can remain united at their ends, thus bearing the appearance of representing a stage of transverse division. A strong support in favor of the

transverse mode of multiplication lies in the formation of a very long thread consisting of several sections united together by means of a delicate bridge between them. This phenomenon is of common occurrence in any spironema or treponema culture. It is highly probable that the usual mode of division in culture is transverse, although the possibility of longitudinal division cannot be excluded. Recently Meironsky<sup>64</sup> advanced the view that *Spironema* and *Treponema* besides multiplying transversely also do so by a process of fructification (*Doldenbildung*) and budding (*Knospbildung*) similar to that observed in some lower plant organisms. His ideas were chiefly based upon phenomena observed by means of various methods of vital staining, in a culture of *Treponema pallidum* (furnished by Sowade). He describes numerous granules collected in a group at one point or another along the body of the pallidum and also branching out of sprouts from some of the specimens. There are many factors to be taken into consideration in such an experimental arrangement which will make it difficult to properly estimate the value of the observations. Those made under the microscope on a preparation containing the organisms, consisting of semi-coagulated horse serum, solution of precipitable aniline dyes (effected particularly through a change of reaction in the medium) are of a disputable character when we consider the absence of strict aseptic precautions as well as the comparatively long period of observation (many days and weeks) during which a preparation had been kept for observation. It is possible that under these unfavorable conditions various forms of involution result which do not appear under normal cultural conditions. Certainly it is not convincing that this so-called fructification or budding also occurs in the body of infected hosts.

Balfour<sup>65, 66</sup> noticed the appearance of certain granules within some of the erythrocytes of fowls which had just stood the first attack of the Sudanese fowl spironematoses and thought that these granules give rise to a new generation of the spiral forms of the organism which reappear at the second attack. That is to say, that a *Spironema* found by Balfour in a Sudan epizootia possesses a spiral and a granular phase of life. Leishman,<sup>67</sup> Blanc,<sup>68</sup> Fantham,<sup>69</sup> Nuttall,<sup>69a</sup> and Hindle<sup>70</sup> also entertain the belief that *Spironema duttoni* and *Spironema gallinarum* adopt a granular form under certain conditions, and that a spiral form can sprout out when the conditions become favorable. Thus in the body of infected ticks these spiral organisms undergo segmentation and numerous granules are produced, a process analogous to sporulation. These granules were called by the authors coccoid bodies, infective granules, or spores. This view was supported by the histological studies of Hindle who secured a series of preparations in which these granules can be demonstrated in the body of the tick. According to Hindle these granules become spiral when the infected tick is incubated at 37° C. for a certain time. In contradistinction to the above findings, Marchoux and Couvy,<sup>71</sup> Gleitsmann,<sup>72</sup> Gonder,<sup>45</sup> and Todd and Wolbach<sup>73</sup> maintain that in an infected tick some motile spironemata can always be demonstrated and that the granules described by Hindle and others are not specific for the infected ticks, but can also be found in the control specimens. Fantham<sup>69</sup> points out, however, that the granules of normal ticks are not identical with the coccoid bodies of *Spironema* found in the infected ticks.

Schaudinn, Prowazek, and others noticed that certain species formed nodules under adverse conditions and suggested that these may represent a resting stage (or resistant form); but Schellack and Wolbach regard them as a depression phenomenon which can also be induced by prolonged treatment of the organisms with a saline solution. Besides, there is a peculiar, highly refractile, round body which is very often found attached somewhere along the side of the body of the organism. There may be one or more such bodies in a specimen. The significance of this body is still obscure, but it may possibly be caused through a disturbance of the osmotic equivalence existing between the cytoplasm of the organism and the medium, not unlike the phenomenon known as plasmoptysis. I have demonstrated its occurrence in the cultivated specimens of various species of *Spironema* and *Treponema*. The body is more frequently present in an old culture in which innumerable granules are also found. In certain culture tubes these minute granules are mostly of varying size. By making a transplant of such a culture into a new medium it was found that, when examined several days later, the new culture contained many short spiral forms which were in one manner or another intimately connected with the granules. This phenomenon suggested the possibility of representing the sprouting of the spiral forms from the granules.

#### PATHOGENICITY.

Spironemata and treponemata are parasitic, and some varieties are responsible for various diseases in man and animals. Various forms of acute febrile diseases, as well as chronic pathological conditions are caused by the invasion of the blood or tissues by this group of organisms. It may be mentioned that the spironemata are almost always transmitted from a sick individual to a normal person through the intermediary of certain blood-sucking insects and invade the blood principally, whereas the pathogenic treponemata are carried from man to man by direct contact and show a predilection for various organs and tissues. As a rule the phase of the spironemal infection is acute and brief and that of the treponemal invasion runs a chronic course, as instanced in the former case by the type of relapsing and tick fevers and in the latter by syphilis and yaws.

Besides the pathogenic species there are a large number of saprophytic varieties belonging to these two genera (or one, according to certain classifications) which are common inhabitants of the oral cavity, genitalia, and alimentary tract of man and animals. Some forms are frequently associated with certain pathological conditions, but their etiologic significance has not been definitely determined. Such is the case with *S. balanitidis* in *Ulcus erosina circinata*, *S. vincenti* in an acute angina, *S. schaudinni* in *Ulcus tropicus* and *Treponema mucosum* in pyorrhea alveolaris, etc. It may be that some of these play the rôle of a secondary invader and aggravate the conditions.

In the following table, I have enumerated the different species of *Spironema* and *Treponema* which have hitherto been observed by various investigators throughout the animal kingdom. It will be seen that the search has been more thorough in the case of the warm-blooded vertebrates than the cold-blooded orders, while even mosquitoes, ants, mites, and fleas are found to harbor certain species of these organisms.

## SPIROCHÆTÆ (LARGE FREE-LIVING FORMS).

- Sp. plicatilis.\* Fresh water. 100-200 $\mu$ , 500 $\mu$  max.  $\times$  0.5-0.75 $\mu$  Ehrenberg, 1838.  
 Sp. plicatilis marina\*.....Zuelzer, 1912.  
 Sp. plicatilis eurystrepta\*.....Zuelzer, 1912.  
 Sp. stenostrepta\* .....Zuelzer, 1912.  
 Sp. daxensis† .....Hotspring. 30-100 $\mu$   $\times$  0.5-0.75 $\mu$  Cantacuzene, 1910.

\*Zuelzer cultivated these varieties in a suitable medium and proved each of them to be different from the others; hence she made subspecies. Schaudinn considered them to represent male and female forms.

†In the water of hot springs of Dax (52°-56° C.).

## CRISTISPIRÆ AND SAPROSPIRÆ (LARGE SAPROPHYTIC AND COMMENSAL FORMS IN THE ALIMENTARY CANALS OF SHELLFISH).

- Cristispira balbianii\*....Ostrea angulata  
   O. edulis.....100-120 $\mu$   $\times$  3-5 $\mu$ . Certes, 1882.  
 C. anodontæ.....Fresh water mussel,  
   A. cygnea; also  
   A. mutabilis. 130 $\mu$   $\times$  3-4 $\mu$ .....Keysseltz, 1906.  
 C. spiculifera†.....ditto.....28-36 $\mu$   $\times$  0.7-1.1 $\mu$ . Schellack, 1909.  
 C. primæ††.....Prima squamosa,  
   P. nobilis.....10-60 $\mu$   $\times$  0.5-3 $\mu$ ....Gonder, 1908.  
 C. mactræ.....Mactra sulcataria 45-70 $\mu$   $\times$  0.8-1.0 $\mu$ ..Prowazek, 1910.  
 C. pectinis.....Pecten jacobæus..72 $\mu$      $\times$  1.5 $\mu$ .....Gross, 1910.  
 C. interrogationis.....25 $\mu$      $\times$  0.5 $\mu$ .....Gross, 1910.  
 C. veneris.....Venus casta.....Dobell, 1910.  
 Saprospira grandis.....Gross, 1912.  
 S. nana.....Gross, 1912.

\*Once considered to be trypanosome or spirochæta.

†Bosanquet doubts its being a separate species from C. anodontæ.

††Gonder once described a blepharoplast near one blunt end; nucleus in single rod or irregular masses. Specimens with rod-formed nucleus may be male elements, since they are highly active, the others female or indifferent elements. A concentration of all the chromatin into one rounded mass was sometimes observed. Encystment also occurs. Gonder no longer upholds his above-cited interpretations, explaining them on the ground of faulty technical handling of the preparation.

(SHELLACK)		Length		Length		Ends.
		Average.	Extremes.	Average.	Extremes.	
C. balbianii.....	Ostrea edulis.....	39	35-42	1.3	1.1-1.5	Rounded, no t. ap.
C. ostræ.....	Ostrea edulis.....	41.5	38-42.5	1.1	1.0-1.3	Sharp, no t. ap.
C. chamæ.....	Chama gryphoides } Ch. sinistrorsa }	45.6	45-46.5	1.4	1.3-1.5	Rounded, no t. ap.
C. anodontæ....	Anodonta mutabilis	46	39-50.5	1.0	0.9-1.2	Rounded, no t. ap.
C. spiculifera....	Anodonta .....	33	28-36.5	0.9	0.7-1.1	Pointed, t. filam.
C. modiolæ.....	M. barbato.....	37.5	36-40	0.8	0.7-0.9	Rounded, no t. ap.
C. primæ.....	P. nobilis.....	30.4	29-31	1.0	0.8-1.1	Rounded, no t. ap.
C. limæ.....	L. inflato, L. hiano.	37	35-41	1.4	1.0-1.8	Rounded, no t. ap.
C. cardii papillose.	C. papillosum.....	19.1	18.5-20	1.2	1.1-1.4	Rounded, no t. ap.
C. tapetos.....	T. decussata.....	34.5	29-35	1.3	1.1-1.4	Rounded, occasional t. ap.
C. acuminata....	Tapes læta.....	37	43.5-49.5	1.0	0.9-1.1	Pointed, no t. ap.
C. saxicavæ.....	Sax. arctica.....	31	30-32	1.7	1.6-1.8	Rounded, no t. ap.
C. gastrochænæ...	G. dubia.....	29	constant	1.2	1.1-1.3	One end blunt, one sharp, no t. ap.
S. pusella*.....	Anodonta. Umo, Lima, Tapes, etc.	13	12-14	..	0.3-0.4	Sharp pointed.

\*Bosanquet found a spirochæta 10-12 $\mu$  in length which he thinks may be identical with Spirochæta hartmanni of Gonder or with S. pusella of Schellack. No crista?



## SPIRONEMA.

- S. obermeieri*\*.....Man, Europe.  $8-16\mu \times 0.25\mu$ .....Cohn, 1877.<sup>74</sup>  
*S. carteri*.....Man, India.  $8-16\mu \times 0.2\mu$ .....Mackie, 1907.<sup>75</sup>  
*S. duttoni*.....Man, West Africa.  $16-30\mu \times 0.2\mu$ .....Novy and Knapp, 1906, Breinl, 1906.<sup>76</sup>  
*S. kochi*.....Man, East Africa.....Schellack, 1907.<sup>77</sup>  
*S. berbera*.....Man, Algiers..... $12\mu$ .....Sergent, 1908.<sup>78</sup>  
*S. ægyptica*.....Man, Egypt..... $13.5\mu$ .....  
*S. novyi*.....Man, North America..... $12\mu$ .....Schellack, 1907.<sup>77</sup>  
*S. ictero-hemor-*  
*rhagæ*.....Man,  $4-9\mu \times 0.3\mu$ , exceptionally  $25\mu$ .....Inada, 1914-15.<sup>79</sup>  
*S. nodosum*.....Man.....Huebener and Reiter, 1916.<sup>80</sup>  
*S. gallinarum*†.....Fowl.....Marchoux and Salimbeni, 1903.<sup>81</sup>  
*S. anserina*.....Goose.....Sacharoff, 1890.<sup>82</sup>  
*S. theileri*.....Cattle,  $20-30\mu \times 0.25-0.33\mu$ .....Läveran, 1902.  
*S. bovis cafferis*.....Cattle.....Nuttall, 1910.  
*S. equi*.....Horse.....Novy and Knapp.  
*S. equina*.....Horse.....Theiler, 1906.<sup>83</sup>  
*S. ovina*.....Sheep.....Blanchard, 1906.  
*S. macaci*.....Inacacus, Ceylon.....Castellani and Chambers, 1908.  
*S. pitheci*.....Cereopethicus pates.....Thioux and Dufongère, 1910.  
 French Sudan.....  
*S. lutræ*.....Otter.....Prowazek, 1907.  
*S. lovati*.....Grouse's coccum  $16-35.5\mu \times$ .....Fantham, 1910.  
*S. vesperuginis*.....Tunisian bat.  $12-18\mu \times 0.25\mu$ .....Gonder, 1908.  
*S. lagopodis*.....Grouse's blood... $10-18\mu \times$ .....Fantham, 1910.  
*S. laverani*.....Mouse  $1.8-3.75\mu \times 0.1-0.2\mu$ .....Breinl and Kinghorn, 1906.<sup>84</sup>  
*S. suis*.....Pigskin lesion or tumor  $6-12\mu$ .....Dodd, 1906, Cleland, 1906.  
*S. muris*.....Rat..... $3-7\mu \times 0.2\mu$ .....Wenyon, 1906.<sup>85</sup>  
*S. minor*.....Rat..... $5-9\mu$ .....Carter, 1887.<sup>86</sup>  
*S. microgyratum*.....Ulcerated cancers,  $5-11\mu \times 1.5-2\mu$   $2.5-6\mu \times 0.16-0.25\mu$ .....Löwenthal, 1906.<sup>87</sup>  
*S. eugyratum*.....Human intestine, stenogyr.  $4.6-7.3\mu$ .....Werner, 1906.  
*S. stenogyratum*.....Human intestine,  $3.6-6.7\mu$ .....Werner, 1906.  
*S. gondii*.....Rodent Ctenodactylus gondi  $16-19\mu \times 0.3\mu$ .....Nicolle, 1907.  
*S. gadi*.....S. W. Fish, Gadus minutus.....  
 $10-16\mu \times 3.5-4\mu$ .....Neumann, 1909.  
*S. pelanchysis* S. W. Pelamys sarda..... $9-10\mu \times 1-1.9\mu$ .....Neumann, 1909.  
*S. jonesii*, F. W. Fish, Clavias angolensis  $18\mu \times 0.1\mu$ .....Dutton, Todd and Toby, 1906.  
*S. hartmanni*.....Prima squamosa,  
 P. nobilis intestine  $6-14\mu \times 1\mu$ .....Gonder, 1908.<sup>88</sup>  
*S. bufonis*.....Bufo vulgaris Rectum  $8-10\mu \times 1.5\mu$ .....Dobell, 1908.  
*S. minei*.....Work. Ants. Termes lucifugus  $15-50\mu \times 0.3-1\mu$ .....Prowazek, 1910.  
*S. glossinæ*.....Tse-tsefly stomach  $8-15\mu$ .....Novy and Knapp, 1906.  
*S. culicus*.....Gnat. aliment. canal large.....Jaffe, 1907.  
*S. buccalis*††..... $12-20\mu \times 0.5-1\mu$ .....Cohn, 1877.  
*S. vincenti*.....Pharyngitis..... $10-40\mu$ .....Blanchard, 1906.<sup>90</sup>  
*S. gracilis*.....Abscess near jaw.....Vesprémi, 1907.<sup>91</sup>  
*S. schandlini*.....Tropical ulcer.....Prowazek, 1907.<sup>92</sup>  
*S. pseudopallidum*.....Various ulcers.....Mulzer, 1905.<sup>93</sup>  
*S. bronchialis*.....Bronchitis in Ceylon  $15-30\mu$ .....Castellani, 1907.<sup>94</sup>  
*S. phagedenis*.....Phagedenic ulcer in man.....Noguchi, 1912.<sup>95</sup>  
*S. refringens*..... $8-12\mu \times 0.33\mu$ .....Schandlin, 1905.<sup>96</sup>  
*S. balanitidis*.....Balanitis..... $8-12\mu \times 0.5-0.75\mu$ .....Hoffmann and Prowazek, 1906.<sup>97</sup>  
*S. obtusum*.....Yaws lesion.....Castellani, 1905.<sup>98</sup>

## SPIRONEMA (CONT'D).

<i>S. acuminatum</i> .....	Yaws .....	Castellani, 1905. <sup>96</sup>
<i>S. aboriginalis</i> .....	Ulcerative granuloma on pedenda....	
	.....	18-20 $\mu$ .....Cleland, 1909. <sup>97</sup>
<i>S. interrogans</i> .....	Yellow fever .....	14 $\mu$ $\times$ 0.17 $\mu$ .....Stimson, 1909.
<i>S. hyos</i> .....	Hog cholera .....	King, Hoffmann, Bæslack, 1913. <sup>98</sup>
		99, 100
<i>S. grassi</i> .....	Termite in Italy.....	Doflein.
<i>S. termitis</i> .....	Termite in Ceylon large.....	Dobell, 1910.
<i>S. ctenocephali</i> .....	Dog flea .....	Patton.

\*Synonymous with *S. recurrentis*, Lebert, 1874.

†There are three subspecies: *S. granulosa* penetrans, in Sudan; *S. nicolli* in Tunis, and *S. neveuxi* in Senegal.

‡‡Subspecies: *Undulata* and *inequalis*.

Lingard described *Spirosonema* in the blood of the camel, dog, elephant and horse; James, in an ulcer of the dog's muzzle, and Lucet in the gastro enteritis; Mathias and Leger in the blood of the zebra and antelope; Bell and Ruquet in the stomach of a normal dog; Dobell in *Tropidonotus stolatus*; Mühlens and Gleitmann in the boa constrictor; Ed. and Ét. Sergent in the alimentary tract of *Anopheles maculipennis* larva; Mühlens often found spirochaetes resembling *S. recurrentis* in the *Culex* mosquitoes.

## TRYPONEMA.

<i>T. pallidum</i> .....	Syphilis .....	6-14 $\mu$ $\times$ 0.2-0.25 $\mu$ ....Schaudinn and Hoffmann, 1905. <sup>6</sup>
<i>T. pertenuis</i> .....	Yaws .....	Castellani, 1905. <sup>101</sup>
<i>T. microdentium</i> .....	.....	3-12 $\mu$ $\times$ 0.2-0.25 $\mu$ ....Noguchi, 1912. <sup>102</sup>
<i>T. dentium</i> †.....	.....	Koch, 1877.
	‡Subspecies: <i>S. recta</i> , <i>S. tenue</i> , <i>S. denticola</i> .	
<i>T. macrodentium</i> .....	.....	6-18 $\mu$ $\times$ 0.3-0.5 $\mu$ ....Noguchi, 1912. <sup>102</sup>
<i>T. mucosum</i> .....	Pyorrhea alveolaris .....	3-12 $\mu$ $\times$ 0.2-0.25 $\mu$ ....Noguchi, 1912. <sup>103</sup>
<i>T. calligyrum</i> .....	Condyloma .....	Noguchi, 1912. <sup>104</sup>

The foregoing list may be classified according to the habitat of the organisms. Thus, when they multiply within the blood circulation of man or animals they may either lead to a grave pathological condition or may produce no appreciable disturbance of the host that harbors them. In the case of certain tissue-invading species, serious pathological consequences may ensue or the host may remain more or less indifferent to the parasite.

## 1. Varieties which invade the blood principally.

(A) *Those which cause characteristic fevers known as relapsing or tick fever (pathogenic).*—There are seven for man, five for mammals and, at least, two for birds. These are:

*For man:* *Spirosonema obermeieri* (in European relapsing fever), *S. carteri* (in East Indian relapsing fever), *S. duttoni* (African tick fever), *S. kochi* (in East African tick fever), *S. noznyi* (in American relapsing fever), *S. aegyptica*, and *S. berbera* (in Egyptian and North African relapsing fever).

*For mammals:* *Spirosonema theileri* (in South African cattle fever), *S. equi* and *S. equina* (in horse), and *S. ovinæ* (in sheep), *S. macaci* and *S. pitheci* (both in East Indian monkeys).

*For birds:* *Spirosonema gallinarum* (in South American and African chicken fevers), *S. anserina* (in goose fever).

(B) *Those which do not seem to produce any grave condition, but are incidentally found (nonpathogenic).*—

*For man:* None.

*For mammals:* *Spirochæma lutrac* (in otter), *S. gondii*, *S. vesperuginis* (Tunician bat), *S. muris*, *S. minor* (both in rats), *S. lazeari* (in mouse). The organism found by Macneal<sup>105</sup> may be identical with *S. muris*.

*For birds:* *Spirochæma lagopodis* (in grouse's blood).

*For reptiles:* Spirochæmata found in *Tropidonotus* and *Boa*.

*For fish:* *Spirochæma gadi*, *S. pelamydis*, *S. jonesi*.

## 2. Varieties which invade the tissue principally.

(A) *Those which cause characteristic lesions and symptoms (pathogenic).—*

In this group there are no Spirochæma, but the only two known varieties belong to Treponema. No pathogenic tissue parasite belonging to Spirochæmataceæ was found in animals. The two pathogenic treponemata for man are *Treponema pallidum* (in syphilis), and *T. pertenue* (in yaws).

(B) *Those which do not seem to cause any noticeable lesion.*—To this belongs a Spirochæma (or Treponema) discovered by Gaylord<sup>106, 107</sup> and Borrel<sup>108</sup> in mouse cancers. Similar organisms were found by Tyzzer,<sup>109</sup> Deetjen,<sup>110</sup> and Mezinescu.<sup>111</sup>

## 3. Varieties which invade both the blood and the tissues indifferently.

*Spirochæma (?) icterohæmorrhagic* (in Weil's disease prevalent in Japan) and *S. nodosum* (in Weil's disease prevalent in Germany) are the only ones so far known to come under this heading. The former, first discovered by Inada, is probably identical with *S. nodosum* of Huebener and Reiter, who also found it independently of Inada a year later. Stokes confirmed the work of Inada on the cases prevalent in Flanders.\*

## 4. Varieties which may be associated with certain pathological conditions and some of which are regarded as having a more intimate relation to the lesion than that of mere secondary invaders.

There are about seven Spirochæmata and one Treponema which have been recorded in man and may be included in this category. These are: *Spirochæma vincenti* (acute pharyngitis), *S. schaudinni* (in tropical ulcers), *S. bronchialis* (in pulmonary gangrene), *S. balanitidis* (Ulcer erosiva circinata), *S. gracilis* (ulcerating jaw), *S. aboriginalis* (ulcerative granuloma), *S. phagedenis* (in phagedenic ulcer). The only Treponema which may be constantly associated with pyorrhæa alveolaris is *T. mucosum*. *S. suis* was found in the ulcerating lesions of pigs.

## 5. Varieties which are found in or about the body cavities, alimentary tract, and genitalia are nonpathogenic and numerous.

For man there are described eight species of Spirochæma and five species of Treponema. Some of them have been known for many years while others are of recent addition. It may here be noted that similar flora are encountered in mammalian animals. These are as follows:

*Spirochæma refringens*, *S. microgyratum*, *S. buccalis*, *S. acuminatum*, *S. obtusum*, *S. pseudopallidum*, *S. eugyratum*, *S. stenogyratum*, and *Treponema macrodentium*, *T. medium*, *T. microdentium*, *T. dentium*, *T. calligyrum*.

*S. hyos* discovered by King and Baslack<sup>98</sup> in the blood of pigs suffering from hog cholera is considered by them to be the cause of this disease.<sup>99, 100</sup> This or-

\*Personal communication from Dr. Adrian Stokes, Captain R. A. M. C.

ganism should be more extensively studied, particularly in its relation to various spirochaetes found in certain conditions in this animal.<sup>112, 113</sup>

The *Spironema* flora in birds, reptiles, fish and amphibia is practically unexplored, but we find one *Spironema bufonis* in a toad.

For insects there are on record several spironemata; namely, *S. culcis* (culex mosquito), *S. termitus*, *S. grassi* (both in mites), *S. ctenocephali* (in dog fleas).

Before leaving this chapter it may be well to dwell somewhat more extensively on the two recently discovered pathogenic spironemata. Brief mention has been made of the one, namely Inada's *S. icterohemorrhagiae*, causing Weil's disease. The other, discovered by Futaki, Takaki and Taniguchi<sup>114</sup> in the blood and glandular tissues in two cases of rat-bite fever in Japan, is a *Spironema* believed by them to be allied to *S. recurrentis*.

Since Weil<sup>115</sup> called attention to the existence of an infectious disease characterized by a sudden onset, chills, high fever, muscular pains, jaundice, occasional hemorrhages in the skin, and acute nephritis, there have appeared numerous contributions to establish the entity of the disease. It has been found to break out among the soldiers in a barrack or among butchers or sewage-drainers. The mortality in European epidemics was rather low (about 15 per cent). The disease reaches its maximum on the 9th or 10th day, and then gradually ends in a lysis which retards the recovery of health for about a month or longer. Death occurs before the end of the second week of the illness. A similar disease has been known in Japan for the past several years. It was reported in certain districts (Kiushiu, Chiba, Shikoku) to have claimed many thousands of victims every year among the farmers, miners, and other laborers. Children under ten years of age are seldom affected, while those who are more occupied in field work contract the disease more frequently. There seems to be no authentic instance in which the disease was carried to another individual by direct contact. While many bacilli and cocci had been isolated and temporarily held to be the causative agent of this disease, no conclusive evidence had been adduced to support them. Jaeger<sup>116</sup> once described a *B. proteus fluorescens* as the cause of Weil's disease, prevalent in 1892.

Since 1912 Inada and his associates<sup>79</sup> have undertaken an extensive experimental study of this disease, and in 1914 succeeded in transmitting it to guinea pigs. Macacus, rabbits, rats and mice were partially or completely refractory to the inoculation. The most important points of the work of these investigators are (1) the reproduction of the typical symptoms (fever, jaundice, acute nephritis, swelling, and fatty degeneration of the liver, generalized hemorrhages, subnormal temperature before death, etc.); (2) the fact that successful inoculation of the guinea pig depends upon the period of the disease at which the blood was drawn from the patient; namely, no positive result was obtained with material taken after the first week of illness; and (3) the discovery of the *Spirochæta*, *S. icterohemorrhagiae* in the blood, visceral organs, glands, affected skin, and muscles, both in man and the experimentally infected guinea pig.

It must be mentioned that the discovery of the *Spironema* was first made early in 1915 with the tissues and blood of the guinea pig, as the organisms are more abundant in experimental Weil's disease than in human cases. In October, 1915, an opportunity was afforded me to observe a number of cases of

this disease occurring in Chiba and, through the cooperation of Dr. Miyajima, some material for experimental studies was collected. One of the patients had had the attack a month previously and was at the convalescent stage. He was anemic, thin, and moderately jaundiced. The urine (dark, turbid) was collected and inoculated into the peritoneal cavity of the guinea pig. The animal started to show the typical symptoms (fever, jaundice, epistaxis, petechia, bile pigment in the urine, etc.) within one week and was examined just before death. The heart's blood showed *S. icterohemorrhagicæ* in moderate numbers. They were motile (their curves were irregular and showed lateral twitching motions or some serpentine movements). Their length varied from  $9\ \mu$  to  $12\ \mu$  and the width was about  $0.4\ \mu$ . More organisms were seen in the emulsions of the liver and kidney. Some of the specimens were as long as  $16\ \mu$  and some as short at  $4\ \mu$ . The number of curves varied from 4 to 10. Inada, Ido and Hoki, and others state that the body of the organism seems to be beaded when examined under the darkfield microscope. Like other minute treponemata or spironemata, the unstained *Spironema* of Weil's disease is invisible under the ordinary microscope. When stained with the Giemsa, carbol fuchsin, genitan violet, or Fontana stains, the organism presents a spiral thread possessing only a few large curves with pointed extremities. There is a certain resemblance to Vincent's *Spirochæta*, although it is somewhat smaller and finer than the latter. A flagellum has not been demonstrated, but in a preparation stained according to the modified Fontana method,\* I was able to see a delicate projection drawn out of the pointed end of the organism. Probably there is a terminal thread. It is quite astonishing, however, to find that the organisms stained by the Levaditi method appear to be very heavy, irregular forms with a few tortuous bends and blunt ends. By applying a modified technic<sup>117</sup> the organisms stain much more elegantly and preserve their delicate appearance.

As will be mentioned later, the *Spironema icterohemorrhagicæ* has been successfully grown on artificial media and the disease reproduced in the guinea pig by means of the pure culture.

Huebener and Reiter<sup>80</sup> reported early last year (1916) that they were also able to find a *Spirochæta* in the experimental Weil's disease in the guinea pig. The *Spirochæta*, designated *S. nodosa* by them, seems to be identical with the strains isolated by the Japanese investigators. As briefly referred to, Stokes has just isolated the same organisms for the Weil's disease existing in Belgium. He also succeeded in reproducing the typical disease in guinea pigs in which the organisms were demonstrated in abundance.

The report of Futaki and his associates on the finding of a spironema in the inflamed skin and lymph glands in two cases of rat-bite fever† is interesting, inasmuch as the clinical feature of this disease had already suggested to Frohn<sup>118</sup> its possible relation to recurrent fever. Hata and others had found

\*Fix air dried film in (1) a mixture of acetic acid 8 c.c., formalin 20 c.c., and distilled water 10 c.c. for a few minutes; rinse off the fixing reagent. Cover the film with (2) a mixture of 20% tannin in 1% phenol, and steam it over a flame for one minute; wash the film well and then immerse the slide in a 0.25% silver nitrate solution for a minute or two. After washing, cover the film once more with (2) and steam it over a flame, wash and dry.

†Symptoms: Incubation of 10 to 27 days, then chills, fever, headache and malaise. Local inflammation at the site of bite; pains in the limbs of the affected side; dark red eruptions and swollen lymph glands; 3 to 7 days fever with an afebrile interval of 2 to 3 days. Temperature curve similar to that of relapsing fever.

an effective therapeutic agent in salvarsan and mercury. These spironemata were found to be actively motile when examined by the darkfield microscope, and were successfully transmitted to the Inus monkey, guinea pig, and white rat for many generations. The organism discovered by Futaki appears to be allied to the blood spironemata of relapsing fevers. In the meanwhile this will raise an interesting question in regard to the possible existence of a spontaneous *Spironema* infection in rats. So far as I am aware, there is no observation on record of the finding of any pathogenic *Spironema* in the rat, notwithstanding the fact that this animal had been much hunted and examined by health officers for the plague bacilli, thus affording numerous opportunities to make an accidental discovery. Perhaps the finding of Futaki may open up a new field wherein to search for a hitherto undiscovered source of disease communicable to man.

#### VIABILITY.

There is a great deal of experimental data bearing upon the viability of various spiral organisms generally, especially upon the most widely investigated species, *Treponema pallidum*. In recording the results it is necessary to make a distinction between experiments made with uncultivated organisms and with those which have already adapted themselves to the artificial cultural conditions, in view of the fact that the latter offer a much greater resistance to certain external influences.

The free living *Spirochæta* lives for about a week or ten days when taken out of its natural habitat and placed in a vessel without the observance of any special precautions. On the other hand, Zuelzer<sup>22</sup> was able to keep various free living species of *Spirochæta* (plicatilis type) alive for an indefinite period of time by keeping them in a hermetically sealed vessel in which a sufficient amount of hydrogen sulphite and certain organic matters derived from stagnant water were supplied from time to time; in other words, in a culture.

The maximum time during which *Cristispira* can be kept alive is about two days even under favorable conditions. No culture has yet been obtained with any member of the shell fish parasites.

For *Spironema* it was found that the pathogenic varieties, including *S. recurrentis*, *S. duttoni*, *S. nocyi*, *S. gallinarum*, still remain infective after a little more than 40 days when kept in a refrigerator (2°-4°C.).<sup>18</sup> At body temperature (37°C) complete disintegration of the organism takes place within 48 hours. No accurate data can be found regarding the saprophytic species which, it may be assumed, can remain alive much longer than their pathogenic congeners.

Of the *Treponema* group, *Treponema pallidum* has received most attention. Authors agree that the syphilis organism quickly becomes sluggish after being removed from the living tissues and that motility can seldom be detected in any specimen which has been maintained at 37°C. for 24 hours. On the other hand, the pallidum contained in a resected tissue (for example, a piece of chancre or rabbit's testicular syphiloma) is still found to be infective after being kept at room temperature or in a refrigerator for 48 hours or sometimes even 72 hours.\* In a culture medium consisting of rabbit's plasma, a piece of rabbit's kidney and

\*Isolated specimens die within 10 hours in a refrigerator (Neisser).

ascitic fluid, many pallida introduced in the form of an emulsion of rabbit's testicular syphiloma remain quite active for 3 or 4 days when kept at 37°C. under anaerobic conditions. But they do not always multiply to form a real culture. It was found that postmortem material containing *Treponema pallidum* may still be able to infect a susceptible animal when inoculated within 24 hours.<sup>119</sup> The organism is killed at a temperature between 50° and 55° C. maintained for twenty minutes.

The resistance and viability of cultivated strains of *T. pallidum* is much greater than that of the organisms found in the tissues. Akatsu, working in my laboratory, found that when the pallidum is isolated from a fluid culture and put in a fraction of a cubic centimeter of the same fluid, it invariably dies within 24 hours, no matter whether it be kept at 37°, 15°, or 2°C., but it survives for 5 days at 37°, 7 days at 15°, and 10 days at 2°C. when kept in 2 c.c. of the fluid. On the other hand, a small portion of a solid culture set aside in a tube remains capable of transplantation into a new medium for 48 to 72 hours at 15°C. and for 1 to 5 days at 2°C. In a quantity of about 2 c.c. of the culture, the organism remains alive as long as twenty days.

In undisturbed cultures *T. pallidum* remains alive for a considerable length of time. Thus a solid culture, set up according to the original method<sup>120</sup> will remain transplantable to a new medium for a period of one year uninterruptedly kept at 37°C. At 15°C. it remains alive after standing 4 or 5 months, while in a refrigerator (2°C.) it survives about 2 months. In a fluid medium consisting of ascitic fluid and a piece of fresh rabbit's kidney covered with fluid paraffin, the organism lives about 2 to 3 months, and in a double tube method<sup>121</sup> about 4 months at 37°C.

*T. calligyrum*, *T. mucosum*, *T. microdentium* are about the same as the pallidum in regard to their resistance and viability. These organisms resist the action of the sun's rays when exposed directly for several hours (4 hours and 30 minutes) at a temperature of 30°C. (summer) and 4°C. (winter), although no growth was obtainable with material exposed for 12 hours (Akatsu).

Drying promptly kills them, that is, no growth can be obtained by transplanting the dried cultures into new media.

The thermal death points for *T. pallidum* as tested out with pure cultures are as follows:

	5 min.	10 min.	15 min.	30 min.	60 min.
45° C.	+	+	+	+	+
50° C.	+	+	+	+	+
55° C.	+	+	—	—	—
60° C.	—	—	—	—	—
65° C.	—	—	—	—	—

The above data were obtained by Akatsu and closely agree with those obtained by Bronfenbrenner,<sup>122</sup> who found that the several strains of *T. pallidum* were destroyed at slightly lower temperatures. It must be stated that Bronfenbrenner used isolated organisms suspended in saline or ascitic fluid, while Akatsu subjected them to the action of heat in a thin culture tube.

## MICROCHEMICAL REACTION.

As mentioned elsewhere, a number of substances have been found to exert a dissolving or disintegrating action upon so-called "spirochaetes" in general as well as upon certain protozoa. This phenomenon is claimed by certain authors to be decisive enough to place the spirochaetes among protozoan organisms, as the majority of bacteria (pneumococcus is an exception) remain unaffected, and some can multiply freely in a saponin solution which destroys spirochaetes. While a too far-reaching generalization from these observations may be avoided, these reagents nevertheless furnish us with an excellent means of studying the microchemical structure of the organisms. The following table contains a summary of all available data which, however, is very fragmentary and incomplete.

TABLE I

	TREPONEMA PALLIDUM.	SPIRONEMA RECURRENTIS.	CRISTISPIRA ANODONTÆ	SPIROCHÆTA PLICATILIS.
Saponin .....	10 per cent solution: 30 min., immobilized, irregular, paler. 1 hour: mostly broken up. Kills in 1:75-000 dilution.	Like pallidum when treated in 10 per cent solution.	10 per cent solution: 1-2 hours, crista fibrillar, and then indistinct.	10 per cent solution: still motile in 30 min.; longer contact makes the body shadowy, but no dissolution.
Sodium taurocholate	10 per cent solution: like the above; kills in 1:2-500 dilution.	10 per cent solution: immobile in 15 min. The outer layer shrinks into irregular masses exposing axial filament. Final disintegration.	10 per cent solution: destroyed in 15 minutes.	Same as saponin.
Sodium glycocholate	Same as sodium taurocholate.			
Sodium cholate....	Same as above, but kills in 1:5,000 dilution.			
Sodium oleate.....	10 per cent solution: Same as above, kills in 1:70,000 dilution.	Almost dissolved in 1 hour, but some may still be motile.		
Cobra lecithid.....	Kills in 1:1,000 dilution.			
Cobra venom.....	Kills in 1:1,000 dilution.			
Pepsin (0.1 in 150 c.c. of 0.3 per cent HCl)	Cells swell up in 2 hours.		Slight change.	Granules appear in or 3 days at 40° C but only slight change at lower temperature.



TABLE I—(CONT'D)

	TREPONEMA PALLIDUM.	SPIRONEMA RECURRENTIS.	CRISTISPIRA ANODONTE.	SPIROCHÆTA PLICATILIS.
Trypsin (0.2 in 10 c.c. of 0.5 per cent $\text{Na}_2\text{CO}_3$ )	Resist the tryptic digestion for many days.	.....	Crista, chambers and contents disappear in 24-48 hours. Membrane resistant.	Granules and axial filament made distinct in short contact. At 40° C., for 2 or 3 days, only the axial filament remains. It may break into many pieces corresponding to curves.
$\text{H}_2\text{SO}_4$ .....	1 per cent solution: Immobilized immediately, shortened, granular, swollen, indistinct curves. 1 hour the same. 30 per cent solution: dissolves the organisms.	1 per cent solution: complete immobilization; many appear thinner, but forms well-preserved. 30 per cent solution: dissolution.	30 per cent solution dissolves them.	1 per cent solution causes immediate stretching of curves, which resume their winding when adding 1 per cent KOH, or vice versa. This can be repeated many times. 30 per cent solution dissolves the spirochæta.
KOH .....	10 per cent solution: rendered indistinct in 30 min.; dissolution in 1 hour.	10 per cent solution: dissolves most of them in 1 hour; more resistant than the pallidum.	1 per cent solution destroys Crista, membrane resists 10 per cent, but dissolved in 30 per cent with heat.	1 per cent solution dissolves granules, 2-30 per cent destroy spirochæta, axial filament most resistant. Treatment with absolute alcohol accelerates the dissolving power of KOH.
$\text{Na}_2\text{CO}_3$ .....	1 per cent solution: immobilized, but no morphological changes.	1 per cent solution: immobilized, slightly granular, but well preserved.	.....	1 per cent solution: no effect on plasma, dissolves granules.

As will be noticed in Table I, certain reagents demonstrate the existence of a resistant membrane in *Cristispira*, a trypsin resistant axial filament in *Spirochæta*, and a shadowy sheath (?) as well as an axial spiral filament in *Spirochæta* and *Treponema*. As in the case of *Spirochæta*, no true dissolution of *Spirochæta* (both *gallinarum* and *recurrentis*) or *Treponema* was effected by the saponin, but after several hours' contact they were shrivelled and broken up into irregular pieces.

*Resistance to Disinfectant and Chemotherapeutic Agents.*—Attempts to determine the resistance of various "spirochætes" are not lacking, but no satisfactory and accurate results were to be expected from the experiments in which their death point had to be determined through the intermediary of susceptible animals. Since the successful cultivation of different "spirochætes" has been effected, it has become possible to determine the effect of different chemicals. The following chart shows a summary of the results obtained in two independent series of experiments by the use of common disinfectants.

## RESISTANCE TO CHEMICALS.

At 37° C.

Lugol kills in	1:3 dil.; 1:5-1:10 in 15 min.; 1:50 not in 1 hour.
Bichloride of mercury kills in	1:5,000 dil.; 1:10,000 in 15 min.; 1:50,000 in 30 min.; 1:100,000 not in 1 hour.

At room temperature

Phenol kills in	1:200; 1:1,000 in 30 min.; 1:5,000 not in 1 hour.
Lysol kills in	1:1,000; 1:5,000 not in 1 hour.
Formalin kills in	1:200; 1:500 in 15 min.; 1:1,000 not in 1 hour.
Potassium permanganese kills in	1:1,000; 1:5,000 in 15 min.; 1:10,000 not in 1 hour.

Turning our attention to the chemotherapeutic agents it is scarcely necessary to remark that, thanks to the pioneer work of Ehrlich and his collaborators, especially to his contribution to our chemical treatment of spironematoses and trypanosomiasis, a new field of scientific research has been inaugurated. Thus Morgenroth initiated a chemotherapy for bacterial diseases by discovering various quinin derivatives as a specific for pneumococcus. Flexner and Clark, with the collaboration of Jacobs and Heidelberger,<sup>123</sup> made an extensive series of experiments in order to discover an effective chemical compound to combat poliomyelitis, wherein they obtained some encouraging results. In their early work they had employed numerous new derivatives of urotropin (hexamethylenetetramine) as this substance was known to penetrate into the intrathecal space. The work has since been extended to various bacterial infections<sup>124, 125, 126, 127</sup> as well as trypanosomiasis and spironematoses (Brown and Pearce) with the use of additional new arsenic and mercurial compounds. While I do not wish to assert that the therapeutic effect of a chemical compound has any direct relation to the latter's disinfecting or sterilizing power against the causative agent *in vitro*, it was nevertheless thought of interest to find out how these new compounds, including various derivatives of urotropin, arsenic, and mercury, would behave in relation to the various species of *Spiroplasma* and *Treponema* in cultures.

It is a well known fact that atoxyl, arsacetin or arsenophenolglycin, and even salvarsan, attack the trypanosomes and spironemata only after being introduced into the body, where they undergo reduction and produce a highly parasitotropic component. Yet, as will be shown in the following table, salvarsan is by no means inactive *in vitro* against *T. pallidum*. It is a fairly powerful treponemicide. Hence it is not without interest to study these compounds *in vitro* and then, when completed, compare the results with their therapeutic effects *in vivo*. The test tube determination of the germicidal property of these substances should form a part of our knowledge in perfecting chemotherapy. With the cooperation of Dr. Jacobs, who is in charge of the preparation of chemotherapeutic agents at the Rockefeller Institute, the following compounds were tested on cultivated strains of *T. pallidum* *in vitro* with the results indicated in the tables. A fuller report will be made later by Dr. Akatsu.

TABLE II.

No.	Preparation.	Concentration	Concentration
		sufficient to kill T. pallidum.	which no longer kills T. pallidum.
9.	<i>p</i> -Bromobenzylhex. chloride	1: 1,000	1: 2,500
16.	<i>o</i> -Xylylenedi-hex. chloride	1: 2,500	1: 5,000
19.	2-Nitro-3,4-Dimethoxybenzylhex. chloride	1: 2,500	1: 5,000
21.	1-( <i>ω</i> -chlorobenzyl)-2-oxy-3-naphthoic methyl ester)+hex.	1: 2,500	1: 5,000
28.	5-Chloromethylvanillin+hex.	1: 750	1: 1,000
29.	5-Chloromethylsalicylic acid+hex.	1: 2,500	1: 1,500
40.	<i>p</i> -iodobenzylbromide+hex.	1: 750	1: 1,000
46.	<i>o</i> -nitrobenzylchloride+hex.	1: 250	1: 500
47.	<i>p</i> -nitrobenzylhex. chloride	1: 750	1: 1,000
50.	Methylhex. iodide	1: 100	1: 250
84.	Chloroacetamide+hex.	1: 1,000	1: 2,500
86.	Oxymethylchloroacetamide+hex.	1: 250	1: 500
90a.	Ethyl bromoacetate+hex.	1: 1,000	1: 2,500
96.	Chloroacetylaniline+hex.	1: 1,000	1: 2,500
97.	$\beta$ -acetoxy- $\alpha$ -chloroacetylnaphthobenzylamine+hex.	1: 1,000	1: 2,500
102.	Chloroacetyl- $\alpha$ -naphthylamine+hex.	1: 500	1: 750
107.	Chloroacetylbenzylamine+hex.	1: 500	1: 750
109.	Chloroacetyl- $\beta$ -naphthylamine+hex.	1: 1,000	1: 2,500
111.	<i>o</i> -Methylchloroacetylbenzylamine+hex.	1: 2,500	1: 5,000
112.	Chloroacetyl- <i>p</i> -aminobenzoic ethyl ester+hex.	1: 1,000	1: 2,500
114.	Chloroacetylurea+hex.	1: 1,000	1: 2,500
121.	Phenoxyethylhex. bromide	1: 250	1: 500
122.	<i>p</i> -Bromochloroacetylaniline+hex.	1: 2,500	1: 5,000
126.	Chloroacetylaminooctolene+hex.	1: 250	1: 500
134.	Chloroacetyl- <i>p</i> -anisidine+hex.	1: 2,500	1: 5,000
138.	Chloroacetylphenylhydrazine+hex.	1: 750	1: 1,000
142.	Chloroacetylhydrazide+hex.	1: 1,000	1: 2,500
146.	Menthyl bromoacetate+hex.	1: 750	1: 1,000
147.	Bromoethylphthalimide+hex.	1: 1,000	1: 2,500
148.	<i>p</i> -nitrobenzoic bromoethyl ester+hex.	1: 250	1: 500
150.	Bromoethyl benzoate+hex.	1: 500	1: 750
158.	$\beta$ -Iodopropionyl- <i>o</i> -anisidine+hex.	1: 1,000	1: 5,000
163.	<i>p</i> -ethoxyphenyl bromomethyl ketone+hex.	1: 500	1: 750
164.	Chloroacetyl- $\psi$ -cumidine+hex.	1: 2,500	1: 5,000
168.	<i>p</i> -Acetamino- $\omega$ -bromoacetophenone+hex.	1: 750	1: 1,000
171.	<i>m</i> -Chloroacetylaminoethylbenzamide+hex.	1: 1,000	1: 2,500
172.	<i>m</i> -Chloroacetyl- $\alpha$ , $\alpha$ -phenylbenzylhydrazine+hex.	1: 2,500	1: 5,000
174.	Chloroacetyl-aminoethyl anisate+hex.	1: 500	1: 750
204.	3-( $\omega$ Bromoacetyl) quinaldine+hex.	1: 2,500	1: 5,000
218.	Tribromo- <i>p</i> -cresyl bromoethyl ether+hex.	1: 2,500	1: 5,000
219.	Chloroacetyl- <i>p</i> -aminoleucomolachite green+hex.*	1: 5,000	1: 7,500
229.	Chloroacetyl- <i>p</i> -aminobenzenazo- <i>p'</i> -dimethylaniline+hex.*	1: 500	1: 750
232.	<i>p</i> -Chloroacetylamino benzeneazo- <i>p'</i> -diethylaniline+hex.	1: 1,000	1: 2,500
234.	$\alpha$ -naphthyl bromoethyl ether+hex.	1: 500	1: 750
239.	<i>o</i> -Acetaminophenyl bromoethyl ether+hex.	1: 1,000	1: 2,500
242.	<i>p</i> -chloroacetylamino diethylaniline+hex.	1: 1,000	1: 2,500
244.	Hex.+chloroacetylaminoethyl <i>p</i> -nitrobenzoate	1: 2,500	1: 5,000
249.	Chloroacetyl- <i>p</i> -aminodipropylaniline+hex.*	1: 500	1: 750
252.	Chloroacetyl- <i>p</i> -aminotetraethyl- <i>p'</i> , <i>p''</i> , -diaminotriphenylmethane+hex.	1: 1,000	1: 2,500
253.	Chloroacetyl diethylaniline+hex.	1: 1,000	1: 2,500
255.	<i>p</i> -Cyanobenzylhex. chloride	1: 1,000	1: 2,500
257.	Chloroacetyl- <i>o</i> -aminophenyl benzoate+hex.	1: 1,000	1: 2,500
261.	Chloroacetyl triphenylmethylamine+hex.	1: 1,000	1: 2,500
262.	Chloroacetyl leucouramine+hex. (*?)	1: 1,000	1: 2,500
263.	Chloroacetyl aminoethyl <i>o</i> -nitrobenzoate+hex.	1: 1,000	1: 2,500
267.	Chloroacetylaminoethyl $\beta$ -naphthoate+hex.	1: 2,500	1: 5,000
271.	Chloroacetyl-N-phenylaminoethyl- <i>p</i> -nitrobenzoate+hex.	1: 1,000	1: 2,500
272.	<i>m</i> -Acetamino- <i>p</i> -tolyl $\omega$ -iodoethyl ketone+hex.	1: 5,000	1: 7,500
273.	Chloroacetyl ethylaminoethyl <i>p</i> -nitrobenzoate+hex.	1: 1,000	1: 2,500
278.	$\alpha$ , $\beta$ -Diphenylchloroacetyl amino-ethanol+hex.	1: 250	1: 500

TABLE II.—(CONT'D)

No.	Preparation.	Concentration sufficient to kill <i>T. pallidum</i> .	Concentration which no longer kills <i>T. pallidum</i> .
280.	Chloroacetyl- <i>m</i> -aminoacetophenone+hex. ....	1: 1,000	1: 2,500
282.	$\alpha$ -Phenyl- $\alpha$ -oxy- $\beta$ -chloroacetylaminioethane+hex. ....	1: 1,000	1: 2,500
283.	<i>p</i> -nitrobenzoylaminoisopropyl chloroacetate+hex. ....	1: 500	1: 750
288.	Iodopropanol+hex. ....	1: 500	1: 750
289.	2-Chloroacetyl-amino-3-oxy-3-methylbutane+hex. ....	1: 2,500	1: 5,000
291.	Chloroacetyl- <i>o</i> -methylphenoxyethylamine+hex. ....	1: 1,000	1: 2,500
293.	Chloroacetyl- $\beta$ -amino-8-butanol+hex. ....	1: 250	1: 500
298.	$\beta$ -Phenyl- $\beta$ -oxy-8-chloroacetylaminopropane+hex. ....	1: 1,000	1: 2,500
301.	$\beta$ -Naphthyl bromethyl ether+hex. ....	1: 1,000	1: 2,500
303.	2-oxy-3, 5-dibromobenzyl bromide (+?) +hex. ....	1: 1,000	1: 2,500†
308.	Chloroacetyl- <i>m</i> -iodoaniline+hex. ....	1: 750	1: 1,000
309.	Chloroacetyl-5-iodo- <i>o</i> -toluidine+hex. ....	1: 750	1: 1,000
M1.	(4-[ <i>p</i> -oxybenzeneazo]-phenylmercuric acetate).....	1: 50,000	1: 75,000
M4.	[ <i>o</i> -loxybenzylideneamino] phenylmercuric acetate+.....	1: 50,000	1: 75,000
M7.	1-Amino-2-[ <i>p</i> -naphthaleneazophenylmercuric acetate]-5-sul- fonic acid .....	1: 25,000	1: 50,000

Hex.=Hexamethylenetetramine.

\*=Grind up in a mortar with a little water and add N/10 HCl carefully until dissolved.

†=Treat as above, using N/10 NaOH instead of HCl.

TABLE III

Names of substances	Concentration sufficient to kill <i>T. pallidum</i> .	Concentration in which <i>T. pallidum</i> survived.
Phenol .....	1: 2,500	1: 5,000
Formalin .....	1: 750	1: 1,000
Lysol .....	1: 5,000	1: 7,500
Sublimate .....	1: 100,000	1: 500,000
Salvarsan .....	1: 7,500	1: 10,000
Neosalvarsan .....	1: 2,500	1: 5,000
Atoxyl .....		
Sodium iodide .....	1: 10	1: 25
Potassium iodide .....	1: 10	1: 25
Lugol's solution .....	1: 75	1: 100
Iodox. benz. acid.....	1: 500	1: 1,000
Trypозofrol .....	1: 25,000	1: 50,000
Nectrypозofrol .....	1: 250	1: 1,000
Sodium cholate .....	1: 5,000	1: 7,500
Sodium glycocholate .....	1: 2,500	1: 5,000
Sodium taurocholate .....	1: 2,500	1: 5,000
Sodium oleicum .....	1: 70,000	1: 50,000
Saponin .....	1: 75,000	1: 100,000
Cholesterin .....	No action	No action
Cobra lecithid .....	1: 1,000	1: 5,000
Cobra venom .....	1: 1,000	1: 5,000

TABLE IV

Names of organisms.	Preparation M1.			Preparation No. 253.	
	1 10,000	1 25,000	1 50,000	1 1,000	1 2,500
<i>T. pallidum</i> , heavy type....	..	—	+	—	+
<i>T. pallidum</i> , thin type.....	—	+	..	—	+
<i>T. calligyrum</i> .....	..	—	+	—	+
<i>T. mucosum</i> .....	..	—	+	—	+
<i>T. microdentium</i> .....	..	—	+	—	+
<i>S. refringens</i> .....	..	—	+	—	+

Table II gives a general survey of these compounds, while Table III puts down the strengths of various well known disinfectants and chemicals for the sake of comparison. Table IV gives the resistance of several culture strains of pallidum and other allied species to the action of two different new compounds.

As briefly mentioned the spironemicidal (or treponemicidal) power of salvarsan and neosalvarsan is alleged to increase considerably when introduced into the living body. In a series of experiments,<sup>122</sup> it was found that by allowing a sterile extract of freshly removed rabbit's liver or defibrinated blood of the same animal to act upon neosalvarsan for three hours at 37°C. the germicidal power of this drug increased from 1:1,000 to 1:2,000 in the case of the liver extract, and from 1:1,000 to 1:5,000 in the case of the blood. The addition of boiled extract had no such activating effect.

*Acquisition of Increased Resistance to Drugs.*—It will be recalled here that the failure of chemotherapy in trypanosomiasis in man and animal is partly due to the production of so-called drug-fast strains of various trypanosomes after the latter have on several occasions been subjected to the action of certain arsenic compounds. These organisms will be destroyed to a great extent by the first injection of the drugs, but if there remain a few which have resisted the first medication, they will multiply and the animal will once more be infested with the organisms. The offspring is more resistant to the action of the same drug than the preceding generation. A large dose of the medicament is necessary to destroy the organisms and to overcome this increased resistance. But as a matter of fact, the increased resistance of the organism to the drug is relatively much greater than that of the infected hosts and the limit will soon be reached beyond which the quantity of the drug cannot be further increased without seriously affecting the infected man or animal.

Experiments of this nature have been made with atoxyl, arsacetin, arsenophenylglycin, etc. To employ Ehrlich's terms, the organotropic affinities of these drugs were so close to the parasitotropic, that it was impossible to employ a sufficient quantity to completely sterilize the infected body, since the administration of such a quantity would mean death or the serious impairment of some of the functions. Ehrlich's conception of a specific chemotherapy was based upon the fact that different cell groups are provided with their characteristic receptor apparatus (chemoceptor), to which a given chemical molecule attaches by means of its side chains. Thus, for trypanosomes there are certain receptors which will fit in with a certain atom complex of atoxyl, arsacetin, etc., while of the infected hosts the organs show much less affinity for them. In developing chemotherapy for syphilis, Ehrlich finally evolved a compound in which the spirone-matropic atom complexes were far more in excess than the organotropic groups. This compound, as is universally known, is dioxydiamidoarsenobenzol, better known as salvarsan. According to Hata<sup>128</sup> the ratio of the dosis curativa and dosis tolerata of this compound is 1:3 for mice and rats infected with *Spironema recurrentis*, and 1:58 for chickens with *S. gallinarum*, while in the case of experimental chancre in rabbits it is between 1:7-1:10. In these animals Ehrlich's *Therapia sterilisans magna* was achieved as also in cases of relapsing fevers in man. In human syphilis, however, in spite of the most powerful spironemicidal

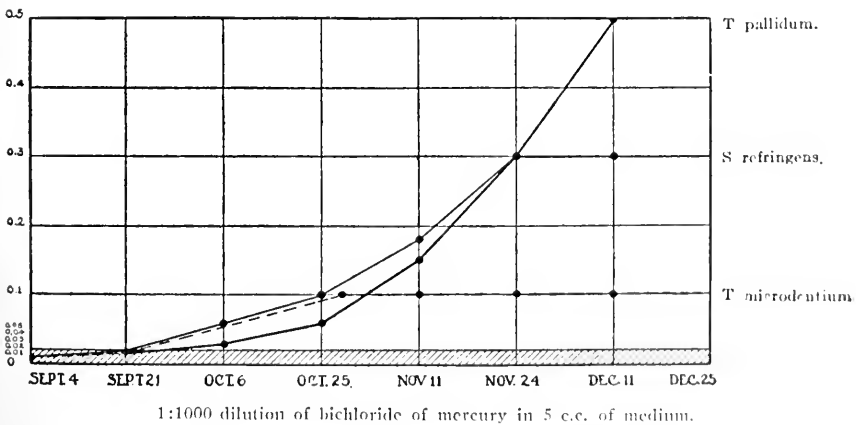
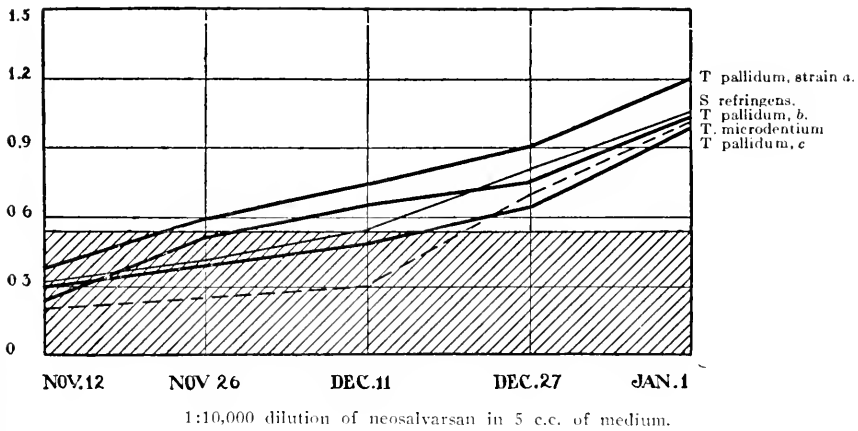
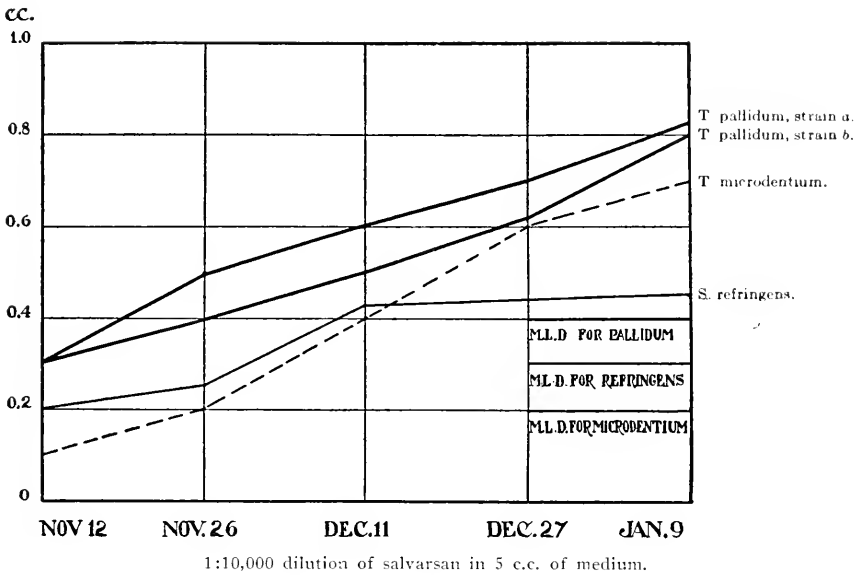
action, his original aim to sterilize the syphilitic body with a single injection of a large dose was not uniformly attained.

Yet there is no doubt that a prompt administration of salvarsan in a sufficient dose during the early stage of infection sterilized the patients, as was evidenced by the increased instances of permanent abortion of the infection and of reinfection after the salvarsan treatment. On the other hand, we are also confronted with repeated recidives in certain patients. We often hear of mercury-resistant as well as salvarsan-refractory cases. It has been known for some time that *Spironema recurrentis* as well as *Spironema duttoni* produces an arsenic-fast strain in mice or rats when the latter are treated with atoxyl, arsacetin, etc. In this respect these spironemata resemble trypanosomes. Marks<sup>129</sup> once considerably raised the resistance of a bacteria to arsenious acid by allowing it to gradually accustom itself to the action of this chemical in test tube cultures. It, therefore, seems not at all improbable that *Spironema* as well as *Treponema* become more resistant to the parasitotropic effect of arsenic compounds and possibly of mercurial salts, not only *in vivo*, but *in vitro*. Akatsu carried out a number of experiments in my laboratory in which he has apparently succeeded in raising to many times their original degree the resistance of the *Treponema* group to salvarsan, neosalvarsan, and bichloride of mercury. The experiments were carried out with cultures of these organisms, the general plan being to cultivate the organisms in media containing these substances in a concentration just short of that required to suppress the growth completely, and to make subcultures from it into new media containing somewhat greater quantities of the chemicals than the preceding series. In the present experiments fluid cultures consisting of ascitic fluid and a piece of fresh rabbit's kidney covered with a layer of liquid paraffin were employed. Subcultures from one medicated culture to another were made at two weeks' intervals, during which time the general condition of the cultures could be estimated. As mentioned above, subcultures are made from tubes still showing numerous actively motile organisms. It is difficult to carry on the culture if one attempts to make a subculture in which too much medicament is present to give a fairly good growth, since no growth will be obtained in a subculture which has been inoculated with a poor culture arrested in its development by an excess of the drugs.

In order to suppress the growth of various treponemata which had not previously been in contact with these compounds, the following doses were found necessary in a total volume of 5 c.c. of the culture medium. The solutions of salvarsan and neosalvarsan were 1:10,000 dilution in water, and bichloride of mercury 1:1,000 dilution. Salvarsan was neutralized with NaOH, as usual.

	SALVARSAN.	NEOSALVARSAN.	HgCl <sub>2</sub> .
Refringens	0.4 c.c.	0.6 c.c.	0.02 c.c.
Pallidum (two strains)	0.375 c.c.	0.5 c.c.	0.02 c.c.
Microdentium	0.2 c.c.	0.3 c.c.	0.02 c.c.

It will be seen from the charts that the resistance of different species of *Treponema* and also of different strains of the same species (*T. pallidum*) seemed to



increase gradually until at the end of ten weeks (five transfers), they were still able to grow very well in a medium which contained several (2 to 3.5) times the quantity of the arsenic compounds originally sufficient to restrain their growth completely. In case of bichloride of mercury the increased rate of tolerance was still more striking within a certain limit of concentration, but there was no further increase in resistance when the medium contained more than 0.5 c.c. of the 1:1000 dilution of this salt. The tissues which usually remain fleshy pink in color for several days became quickly discolored and a dirty grayish black when mixed with the above concentration of  $\text{HgCl}_2$ .

The question of the duration of the acquired resistance to the drugs has not yet been studied a sufficiently long time to draw any conclusions, but the resistance has remained unmodified for at least three generations. It may be mentioned that *Spiroplasma recurrentis* was carried through two generations in mice without undergoing any change in its acquired drug fastness.

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(To be continued.)

## THE EFFECT OF CERTAIN DRUGS UPON SKIN REACTIONS\*

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THAT the oral administration of certain drugs may influence skin reactions to the extent of increasing their degree and severity or producing well marked papular or pustular reactions in the skins of persons who did not react to these injections in preliminary tests, has not been generally recognized until recently.

Amberg and Knox<sup>1</sup> found that the intravenous administration of neutral sodium ortho-iodoxybenzoate and sodium iodosobenzoate inhibited the development of reactions following the intracutaneous injection of serum in sensitized rabbits; sodium iodbenzoate and sodium benzoate in equal molecular concentrations had no inhibitory effect on the local reaction. The authors believed that the inhibitory influence of these drugs was attributable to their effect on oxidative processes. Opposite results have been reported more recently by Sherrick,<sup>2</sup> who found that the administration of potassium iodide either simultaneously, or shortly before or after the intracutaneous injection of luetin and agar, resulted in the production of pustular or nodular reactions in healthy nonsyphilitic persons. Kolmer, Matsunami, and Broadwell<sup>3</sup> confirmed these observations among healthy nonsyphilitic persons, persons sick with diseases other than syphilis and normal rabbits and guinea pigs. The oral administration of potassium iodide may cause the site of a former intracutaneous injection of luetin or agar to "light up" and present well defined inflammatory changes sometimes leading to pustulation as pointed out by Sherrick; we also found that a purer form of luetin prepared of washed spirochetes and free of all culture media, was influenced in a much lesser degree by potassium iodide.

Our studies have been continued to determine the influence of iodides, bromides, chlorides, ether and chloroform upon cutaneous, intracutaneous, and conjunctival tests; also upon the mechanism of the influence of drugs upon skin reactions.

The above mentioned drugs were selected on the basis of the investigations of Jobling and Petersen<sup>4</sup> who claim that the proteolytic ferments of the blood serum are held in check by antiferment in the nature of unsaturated fatty acid radicals and that the latter may be removed by saturation with iodine; also that other halogens and ether and chloroform may remove the antiferment from serum *in vitro* and increase tryptic activity.<sup>5</sup> It is possible that these drugs may influence the local skin reactions in this manner; that is, by increasing tryptic activity and the production of proteotoxins responsible for the lesion. Furthermore we were influenced in selecting the iodides and bromides in this study on the basis of abundant clinical evidence to the effect that their prolonged administration may result in the development of papular and pustular eruptions in the skin.

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## METHOD OF STUDY.

Three intracutaneous tests were conducted, (1) with a luetin prepared after the method of Noguchi and so diluted with sterile salt solution that the proper dose was contained in 0.1 c.c.; (2) with an emulsion of washed *B. prodigiosus*, resuspended in sterile salt solution so that each cubic centimeter contained about two billion bacilli, heated at 60° C. for one hour, preserved with 0.2 per cent tricresol and injected in dose of 0.1 c.c. This preparation has been designated "prodigiosin;" (3) with a 1:10,000 dilution of Koch's old tuberculin injected in

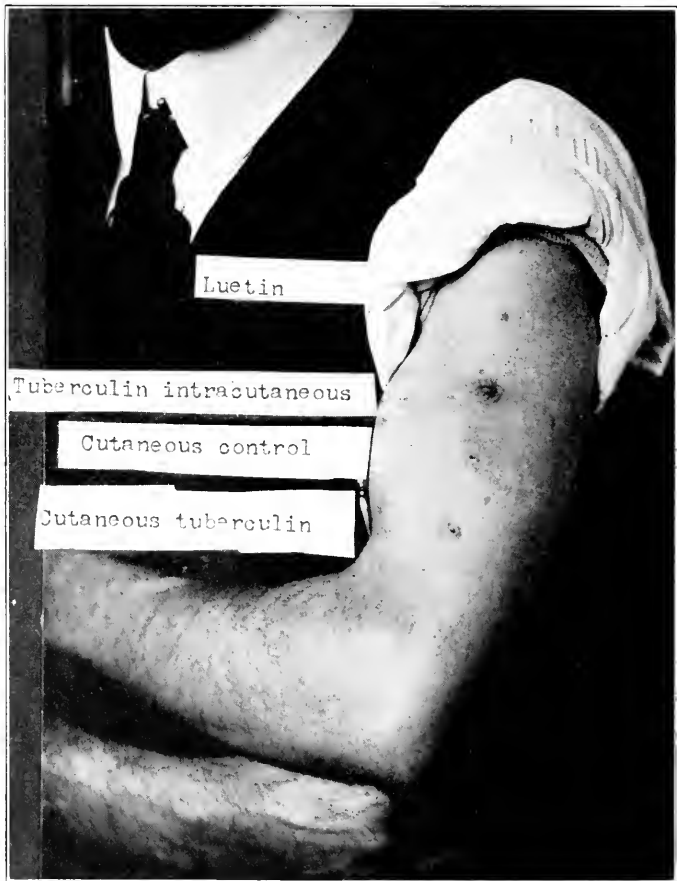


Fig. 1.—Dr. J. A. K.; intracutaneous and cutaneous reactions before the administration of potassium iodide.

amount of 0.1 c.c. Many of the reactions following the injection of this amount of tuberculin were quite severe, so that in a number of tests the amount injected was reduced to 0.05 c.c.

The cutaneous and conjunctival tests were conducted with undiluted and diluted Koch's old tuberculin.

Preliminary skin tests were made among adult persons who either volunteered or gave consent, and the reactions carefully measured and recorded forty-eight hours later. Wassermann reactions were made with the sera of all persons

receiving the intracutaneous luetin and prodigoin tests and with three different antigens; namely, a cholesterinized alcoholic extract of human heart, and alcoholic extract of syphilitic liver and an extract of acetone insoluble lipoids of beef heart.

The majority of persons were Wassermann negative with all antigens; in many persons, as laboratory assistants, medical students, and the authors, syphilis could be excluded with considerable certainty. A number of Wassermann positive and syphilitic persons and persons yielding positive tuberculin reactions



Fig. 2.—Dr. J. A. K.; intracutaneous and cutaneous reactions after the oral administration of 50 grains of potassium iodide.

were purposely included, in order to determine the influence of the drugs under study upon cutaneous and intracutaneous anaphylactic reactions.\*

The drugs were administered by mouth in dose of 10 grains two or three times a day for a period of three days. On the fourth day the skin tests were repeated with the same materials and in exactly the same manner, the reactions being measured and recorded forty-eight hours later.

\*We are indebted to Dr. J. Allen Jackson, Chief Resident Physician in the Department for the Insane, Philadelphia General Hospital, for cooperation and clinical facilities afforded us in the study of these cases.

The majority of persons were kept under observation for a period of ten days or longer to note the retarded effect, if any, of the drugs upon the first and second sets of skin tests.

As a general rule the luetin and prodigiosin tests were conducted together in all persons, the preliminary tests being made in the skin of the left arm and the second tests, in the skin of the right arm.

Both the luetin and prodigiosin produced reactions in the majority of persons in the preliminary tests. As a general rule the former yielded a slight

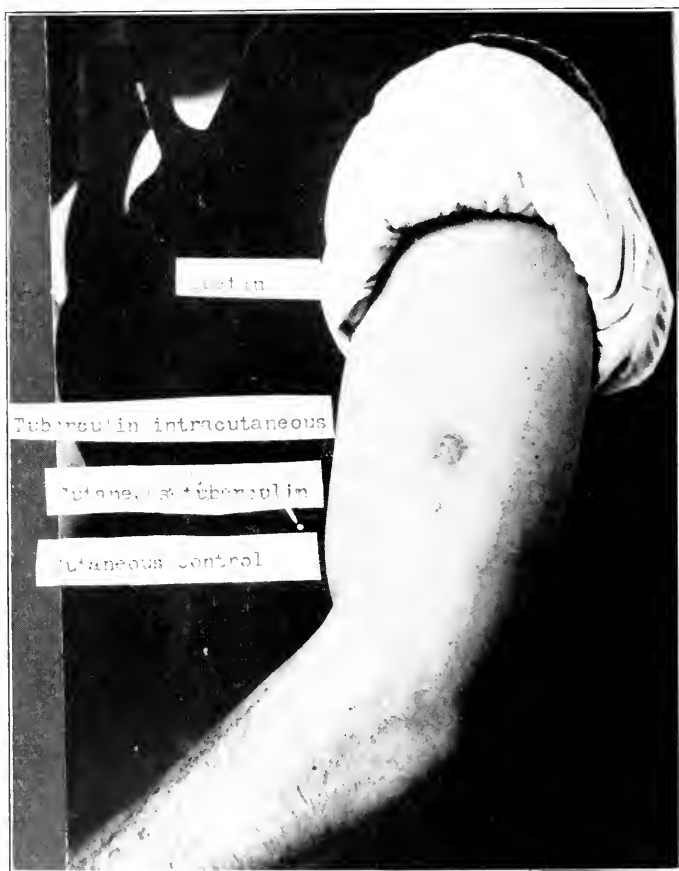


Fig. 3.—Mr. W. G.; intracutaneous and cutaneous reactions before the administration of potassium bromide.

reaction with an area of erythema measuring five millimeters or thereabouts and very slight edema; the prodigiosin reactions were more marked. In determining whether or not a drug had produced an effect we were influenced by the increased area of erythema, the greater infiltration, and frequently the development of a pustule. In each table we have given the size of each area of erythema and an interpretation of the results. Only decided differences in the degree of the reactions were accepted as indicating that the drug under study had increased the severity of the reactions.



## RESULTS.

1. *The influence of potassium and sodium iodide; potassium and sodium bromide; potassium and sodium ammonium chloride; the protiodide of mercury and ether upon the luetin and prodigiosin reactions among Wassermann negative persons.*—The influence of these drugs administered in dose of 10 grains two or three times a day for a period of three days, upon the two intracutaneous tests are shown in Tables I to VI. The protiodide of mercury was included in the series by reason of its wide use in the treatment of syphilis and because the

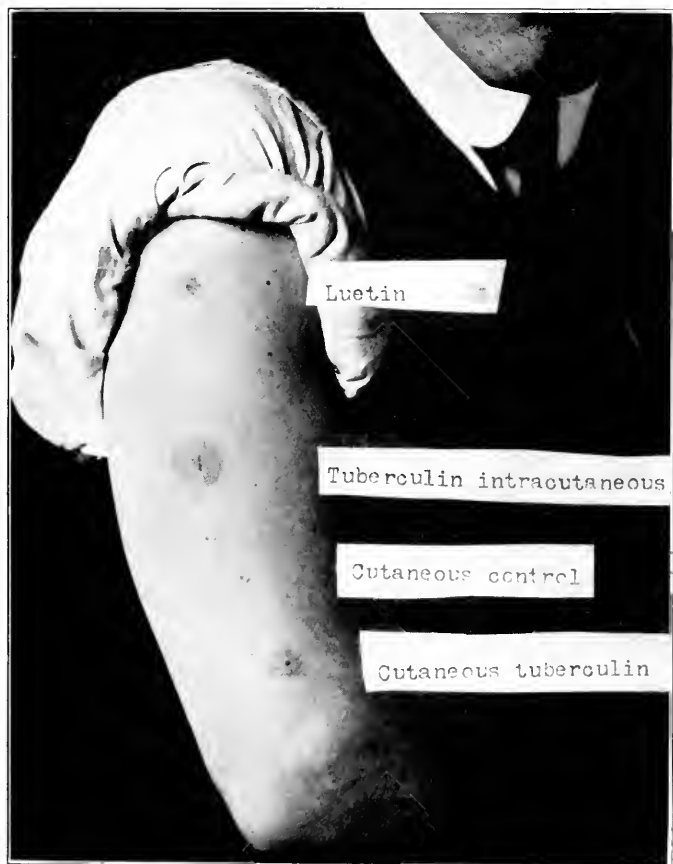


Fig. 4.—Mr. W. G.; intracutaneous and cutaneous reactions after the administration of 60 grains of potassium bromide.

luetin reaction may be employed by the clinician while a patient is taking the drug. The results recorded in Table VII were observed after the administration of  $\frac{1}{4}$  grain four times a day for a period of three days.

The influence of ether was studied by applying the tests before and after anesthesia for surgical operations. The amounts of ether used in the individual cases and the results observed are shown in Table VIII.

TABLE I

THE INFLUENCE OF POTASSIUM IODIDE UPON THE LUETIN AND PRODIGIOSIN INTRACUTANEOUS REACTIONS				
No.	LUETIN REACTIONS		PRODIGIOSIN REACTIONS	
	Before (cm.)	After (cm.)	Before (cm.)	After (cm.)
1.....	0.6×0.5	1.0×0.8	0	0
2.....	0.5×0.5	1.0×0.8	0	0
3.....	0.6×0.6	0.8×0.5	0.8×0.8	1.0×1.0
4.....	0.6×0.4	1.0×0.5	1.0×0.5	1.5×1.0
5.....	0.7×0.7	1.0×0.8	0.7×0.4	2.5×2.0
6.....	0.8×0.7	1.5×1.0	1.5×1.5	2.0×1.5
7.....	0.6×0.6	1.2×1.0	1.5×1.5	1.5×1.5

Results: Luetin reactions influenced in Nos. 1, 2, 4 and 5; prodigiosin reactions influenced in Nos. 4, 5 and 6.

TABLE II

THE INFLUENCE OF SODIUM IODIDE UPON THE LUETIN AND PRODIGIOSIN INTRACUTANEOUS REACTIONS				
No.	LUETIN REACTIONS		PRODIGIOSIN REACTIONS	
	Before (cm.)	After (cm.)	Before (cm.)	After (cm.)
1.....	1.0×1.0	1.0×1.0	1.2×0.8	2.0×1.5
2.....	0.6×0.8	1.0×1.0	0.8×0.6	1.0×0.8
3.....	0.6×0.6	1.0×0.8	0.6×0.8	1.0×0.8
4.....	0.2×0.2	0.2×0.2	0.6×0.4	2.5×2.0
5.....	0.5×0.5	0.5×0.5	0.6×0.6	0.8×0.6
6.....	0.8×0.8	1.2×1.0	1.0×1.0	1.0×1.0
7.....	0.4×0.4	0.6×0.5	0.8×0.6	1.5×1.0
8.....	0.5×0.5	0.8×1.0	1.5×1.0	1.2×1.5
9.....	0.8×0.5	0.7×1.2	1.2×0.6	1.2×1.5

Results: Luetin reactions influenced in Nos. 2, 3, 6 and 8; prodigiosin reactions influenced in Nos. 1, 2, 3, 4 and 7.

TABLE III

THE INFLUENCE OF POTASSIUM BROMIDE UPON THE LUETIN AND PRODIGIOSIN INTRACUTANEOUS REACTIONS				
No.	LUETIN REACTIONS		PRODIGIOSIN REACTIONS	
	Before (cm.)	After (cm.)	Before (cm.)	After (cm.)
1.....	0.6×0.3	0.6×0.5	0.6×0.4	1.0×0.8
2.....	0.9×0.8	1.0×1.0	0.9×0.8	2.0×2.0
3.....	1.0×0.7	1.0×0.5	0.8×0.5	2.5×2.0
4.....	0.7×0.6	0.5×0.5	0.6×0.6	2.0×2.0
5.....	0.8×0.8	0.6×0.6	0.9×0.6	1.0×1.0
6.....	0.2×0.2	0.6×0.6	0	0
7.....	0.5×0.5	0.6×0.8	0	0
8.....	0.1×0.2	0.8×0.6	0.6×0.6	1.0×1.5
9.....	0.6×0.5	0.6×0.6	0.5×0.5	0.5×0.5

Results: Luetin reactions influenced in Nos. 6 and 8; prodigiosin reactions influenced in Nos. 1, 2, 3, 4 and 8.

TABLE IV

THE INFLUENCE OF SODIUM BROMIDE UPON THE LUETIN AND PRODIGIOSIN INTRACUTANEOUS REACTIONS

No.	LUETIN REACTIONS		PRODIGIOSIN REACTIONS	
	Before (cm.)	After (cm.)	Before (cm.)	After (cm.)
1.....	0.3×0.3	1.0×1.5	0.4×0.8	2.0×2.0
2.....	0.5×0.4	0.5×0.5	0.5×0.3	1.0×0.8
3.....	0.9×0.6	0.8×0.5	0.8×0.5	2.0×1.5
4.....	0.4×0.4	0.8×0.5	0.4×0.4	2.0×2.0
5.....	0.8×0.5	0.8×0.5	1.0×1.0	2.0×1.5
6.....	0.4×0.2	0.5×0.3	0.7×0.7	0.9×0.8
7.....	0.4×0.3	0.8×0.8	0.8×0.6	2.0×2.0

Results: Luetin reactions influenced in Nos. 1 and 7; prodigiosin reactions influenced in Nos. 1, 2, 3, 4, 5 and 7.

TABLE V

INFLUENCE OF POTASSIUM CHLORIDE UPON THE LUETIN AND PRODIGIOSIN INTRACUTANEOUS REACTIONS

No.	LUETIN REACTIONS		PRODIGIOSIN REACTIONS	
	Before (cm.)	After (cm.)	Before (cm.)	After (cm.)
1.....	0.1×0.1	0.5×0.5	0.2×0.2	1.0×1.0
2.....	1.0×0.4	0.5×0.5	0.6×0.5	1.0×1.5
3.....	0.6×0.4	0.6×0.5	1.6×0.6	1.5×1.0
4.....	0.5×0.5	0.5×0.5	1.2×0.2	1.0×1.0
5.....	0.5×0.5	0.5×0.5	1.1×1.0	1.0×1.0
6.....	0.6×0.6	0.6×0.5	0.6×0.4	1.0×1.0
7.....	0.5×0.6	0.5×0.5	0	0
8.....	0.5×0.5	0.6×0.6	0	0
9.....	0.5×0.5	0.5×0.5	1.0×0.4	1.0×1.4

Results: Luetin reaction influenced in No. 1; prodigiosin reactions influenced in Nos. 1, 2 and 4.

TABLE VI

INFLUENCE OF AMMONIUM CHLORIDE UPON THE LUETIN AND PRODIGIOSIN INTRACUTANEOUS REACTIONS

No.	LUETIN REACTIONS		PRODIGIOSIN REACTIONS	
	Before (cm.)	After (cm.)	Before (cm.)	After (cm.)
1.....	0.5×0.5	0.5×0.5	0.6×0.6	1.0×1.0
2.....	0.6×0.5	0.5×0.5	0.6×0.5	0.6×0.6
3.....	0.8×0.5	0.5×0.5	0.4×0.4	1.0×1.0
4.....	0.6×0.5	0.5×0.4	0.6×0.6	0.7×0.5
5.....	0.7×0.6	0.5×0.5	1.6×0.6	1.0×0.6
6.....	0.8×0.8	0.5×0.5	1.0×0.6	1.0×1.0
7.....	0.5×0.4	0.6×0.8	0.6×0.5	1.0×1.0
8.....	0.5×0.5	0.5×0.5	0.4×0.4	1.0×1.0
9.....	0.5×0.5	0.4×0.4	1.2×1.0	1.5×1.8
10.....	0.8×0.8	0.8×0.8	2.0×3.0	2.5×3.0

Results: Luetin reactions influenced in none; prodigiosin reactions influenced in Nos. 1, 3, 7 and 8.

TABLE VII

## INFLUENCE OF PROTIDIODE OF MERCURY UPON THE LUTIN AND PRODIGIOSIN INTRACUTANEOUS REACTIONS

No.	LUTIN REACTIONS		PRODIGIOSIN REACTIONS	
	Before (cm.)	After (cm.)	Before (cm.)	After (cm.)
1.....	0.5×0.8	0.5×0.5	0.6×0.5	0.6×0.8
2.....	0.1×0.2	0.4×0.8	1.0×1.0	3.0×3.0
3.....	0.8×0.4	0.5×0.5	0.6×0.4	1.0×1.0
4.....	0.8×0.5	0.5×0.8	0.8×0.8	0.8×0.8
5.....	0.4×0.2	0.3×0.3	0.6×0.3	0.5×0.5
6.....	0.6×0.8	0.6×0.6	0.5×0.5	0.5×0.5

Results: Lutin reaction influenced in No. 2; prodigiosin reactions influenced in Nos. 2 and 3.

TABLE VIII

## THE EFFECT OF ETHER ANESTHESIA UPON THE LUTIN AND PRODIGIOSIN INTRACUTANEOUS REACTIONS

NO.	AMOUNT OF ETHER USED	LUTIN REACTIONS		PRODIGIOSIN REACTIONS	
		Before (cm.)	After (cm.)	Before (cm.)	After (cm.)
1	12 ounces.....	0.5×0.5	0.6×0.6	1.2×1.0	1.0×1.0
2	10 ounces.....	0.7×0.7	0.6×0.6	1.5×1.0	1.2×1.0
3	8 ounces.....	0.7×0.7	0.9×0.8	1.0×1.0	1.3×1.0
4*	15 ounces.....	0.8×0.8	0.8×0.8	1.0×0.8	1.0×1.0
5*	8 ounces.....	1.3×1.0	1.0×0.7	0.6×0.5	0.6×0.4
3*	11 ounces.....	0.5×0.3	0.5×0.5	1.0×0.6	0.7×0.7
7*	16 ounces.....	0.5×0.5	0.7×0.7	0.6×0.4	0.6×0.5

\* Patients received morphine sulphate, gr. 1/4 and atropine sulphate, gr. 1/100, before ether was administered.

Results: Lutin reactions not influenced; prodigiosin reactions not influenced.

A summary of the results is shown in Table IX, which gives the percentage of reactions apparently influenced; that is, rendered more extensive (wider erythema and greater infiltration), by the various drugs.

TABLE IX

## THE EFFECT OF DRUGS UPON THE INTRACUTANEOUS LUTIN AND PRODIGIOSIN REACTIONS AMONG WASSERMANN NEGATIVE PERSONS

DRUGS	TOTAL	PERCENTAGE OF REACTIONS INFLUENCED BY THE DRUG	
		LUTIN	PRODIGIOSIN
Potassium iodide.....	7	71	60
Sodium iodide.....	9	44	55
Protiodide of mercury.....	6	16	33
Potassium bromide.....	9	22	55
Sodium bromide.....	7	28	85
Potassium chloride.....	9	10	33
Ammonium chloride.....	10	0	40
Ether.....	7	0	0
Nitrous oxide.....	3	0	0

As shown in the tables, certain of the drugs used in this study were found to influence the tests by increasing the inflammatory reactions.

a. The prodigiosin reactions were generally more extensive than the luetin reactions and were more readily influenced by certain of the drugs. As previously stated, we have found that the reactions produced by the injection of 0.4 per cent agar-agar were more readily influenced by potassium iodide than the luetin reaction; also that the reaction produced by luetin prepared in the usual manner was more readily influenced than that prepared of washed spirochetes and free of the culture medium (ascites agar). In other words substances most likely to excite inflammatory reactions in the skin appear to be most readily influenced by the iodides and to some extent by bromides.

b. The iodides and particularly the potassium salt, seemed to exert more influence than the other compounds used in this study.

c. The bromides were found to influence these reactions and particularly the prodigiosin reactions, but to a lesser degree than the iodides.

d. The chlorides were found to be practically without effect upon the luetin reactions, although a number of the prodigiosin reactions appeared to be influenced.

e. Nitrous oxide and ether by inhalation had no appreciable influence upon the reactions.

2. *The effect of sodium iodide; potassium and sodium bromide; potassium and ammonium chloride; ether and chloroform upon the intracutaneous luetin and agar reactions in rabbits.*

Preliminary intracutaneous tests (abdominal skin) with luetin and a 0.4 per cent sterile solution of agar-agar, were made in a series of rabbits and the reactions recorded forty-eight hours later.

The drugs were dissolved in water and administered once daily by means of the stomach tube in dose of one grain per sixty kilograms of body weight.

The skin tests were repeated after the third dose and again after six additional doses.

The influence of ether was studied by anesthetizing rabbits for one hour and repeating the luetin and agar tests two hours later; the influence of chloroform was studied in the same manner except that the period of anesthesia was thirty minutes.

The results were indefinite and difficult to read and interpret, due mainly to the difficulty of making an injection into the abdominal skin of a rabbit. As a general rule the reactions to agar were more marked than those excited by the luetin.

In no instance were the reactions influenced by three doses of the drugs. In two animals the reactions were intensified after additional doses of potassium iodide, but the other drugs used in this study were without appreciable effect. The agar reactions were influenced by potassium iodide to a greater extent than the luetin reactions.

Conjunctival tuberculin tests among a series of nontuberculous animals before and after the administration of iodides, bromides and chlorides were negative throughout.

3. *The influence of potassium iodide, potassium bromide and potassium chloride upon cutaneous and intracutaneous tuberculin tests.*—Of 47 adults upon whom the cutaneous tuberculin test of von Pirquet was conducted, six did not show any reaction. Potassium iodide was then administered, the total amount of drug taken varying from 20 to 160 grains, and the tuberculin tests repeated. One person reacted quite distinctly in the second test with "lighting up" of the preliminary test several days later, but we are unable to state whether these effects are to be ascribed to the influence of the potassium iodide or to a delayed tuberculin reaction. A second person presented a mild reaction in the second test.

In a second group of adults the cutaneous and intracutaneous tests were conducted simultaneously on the left arm. All of the reactions were positive and were carefully measured forty-eight hours later; the following drugs were then administered in doses of 10 grains, twice daily for a period of three days: potassium iodide, potassium bromide and potassium chloride. Both tests were then repeated on the left arm and the reactions measured and recorded forty-eight hours later. The results are shown in Table X.

TABLE X

## THE EFFECT OF DRUGS UPON THE CUTANEOUS AND INTRACUTANEOUS TUBERCULIN REACTIONS

NAME	DRUG	CUTANEOUS TUBERCULIN		INTRACUTANEOUS TUBERCULIN	
		Before (cm.)	After (cm.)	Before (cm.)	After (cm.)
Dr. E. S...	Potass. iodide....	1.0×1.0	1.5×1.5	3.0×4.0	3.0×4.0
Dr. J. A. K.	Potass. iodide....	1.6×1.5	3.0×3.0	1.7×1.6	4.1×4.0
Dr. T. M...	Potass. brom....	1.0×1.5	1.0×1.5	2.0×2.0	2.0×1.5
Mr. W. G...	Potass. brom....	0.5×0.5	0.8×1.0	2.5×2.5	2.0×2.5
Dr. S. L...	Potass. brom....	1.0×1.0	2.3×2.0	4.0×4.0	5.0×5.5
Dr. B. M...	Potass. chloride...	1.0×1.0	1.0×1.0	4.0×4.0	4.5×4.5
Mr. C. H.	Potass. chloride...	0.5×0.6	0.8×0.6	2.8×2.5	3.5×3.0

TABLE XI

## EFFECT OF DRUGS UPON THE LUTETIN AND PRODIGIOSIN INTRACUTANEOUS REACTIONS AMONG WASSERMANN POSITIVE PERSONS

NO.	WASSERMANN REACTION	DRUG	LUTETIN REACTIONS		PRODIGIOSIN REACTIONS	
			Before (cm.)	After (cm.)	Before (cm.)	After (cm.)
1	Strongly Positive.....	Potass. iodide.....	1.0×1.2	1.5×1.8	0.7×0.4	0.8×0.5
2	Strongly Positive.....	Sod. iodide.....	1.0×0.6	1.5×1.5	0.8×1.0	2.0×2.0
3	Weakly Positive.....	Protiodid. mercury..	1.0×0.8	1.0×1.0	1.0×1.0	3.0×2.5
4	Strongly Positive.....	Potass. brom.....	0.2×0.4	0.3×0.4	0.5×0.4	1.0×1.0
5	Weakly Positive.....	Sod. brom.....	0.8×0.5	0.6×0.5	1.0×1.0	2.0×1.5
6	Moderately Positive...	Sod. brom.....	1.3×1.2	1.0×1.0	1.0×0.6	1.0×1.5
7	Weakly Positive.....	Potass. chlor.....	1.0×1.0	1.0×1.0	1.0×0.5	1.0×0.8
8	Strongly Positive.....	Ether *	1.0×1.0	1.0×0.8	0.9×0.9	1.0×1.0

\* 10 ounces.

Results: Lutetin reactions regarded as positive in Nos. 1, 2, 3, 6, 7, 8 and influenced in Nos. 1 and 2; prodigiosin reactions influenced in Nos. 2, 3, 4 and 5.

Potassium iodide was found to influence both the cutaneous and intracutaneous reactions of two persons as evidenced by wider inflammatory areolæ the greater edema (Dr. E. S. and Dr. J. A. K.).

Potassium bromide produced a similar influence upon the reactions of one of three persons (Dr. S. I.).

Potassium chloride did not produce any appreciable influence upon the cutaneous reactions of two persons, but increased very slightly the intracutaneous reaction of one of these persons (Mr. C. H.).

It appears that these drugs and particularly potassium iodide, may increase the inflammatory phenomena of an anaphylactic reaction although in these tuberculin reactions the second reactions may have been influenced by the previous tests. Among persons in the tertiary stage of syphilis, presenting positive Wassermann reactions and positive luetin reactions in most instances, potassium iodide appeared to increase the degree of reaction to luetin, while the prodigiosin reactions were influenced to a greater degree (Table XI).

#### MECHANISM OF THE ACTION OF DRUGS UPON SKIN REACTIONS.

Skin reactions may be divided into three varieties, (1) the true anaphylactic reaction due to the interaction in the skin of specific protein antigen and specific antibody; (2) the pseudo or nonspecific protein reaction due to the interaction in the skin of general protein substances and nonspecific proteolysins; (3) the traumatic reaction consequent to the operation; or to the irritant qualities of such substances as preformed bacterial toxins and various preservatives as phenol and tricresol contained in the injected material. One of us (J. A. K.) has discussed elsewhere our present knowledge bearing upon the mechanism and clinical significance of these reactions.<sup>6</sup> Certain drugs, and notably the iodides and to a lesser extent the bromides, may influence skin tests by increasing the severity of the reactions. We are of the opinion that this influence is exerted upon the nonspecific and traumatic reactions rather than upon the specific anaphylactic reaction, although definite proof of this assertion is not at hand. Intracutaneous tests are more readily influenced than cutaneous tests; as a rule the reactions produced by the injection of material of irritant properties are most readily influenced; as for example, the injection of agar-agar or ordinary luetin as compared with a luetin of washed spirochetes alone and free of culture medium. Iodides apparently increase the purely inflammatory and suppurative phases of skin reactions.

One of us (J. A. K.) has also tested the influence of potassium iodide upon the Schick toxin reaction in a few persons; the drug apparently increased the severity of the reactions among persons reacting positively and produced mild reactions among a few persons who possessed an antitoxic immunity of sufficient degree to yield negative toxin tests in the preliminary trials.

Definite knowledge of the mechanism of skin reactions is lacking, but there appears to be sufficient experimental data at hand to indicate that the nonspecific and traumatic skin reactions implicate the proteolytic ferments of fixed tissue cells, leucocytes, and serum, which produce from the injected protein and probably from the protein of the person's own serum a soluble toxic substance responsible in part for the reaction in the skin of erythema, edema, and leucocytic infiltration. If this is correct it would appear probable that the iodides, bromides and to a lesser extent the chlorides, are capable of increasing tryptic activity

by removal of antiferment, after the hypothesis of Jobling and Petersen previously mentioned.

As many of the reactions influenced by potassium iodide become pustular with extensive infiltration of the tissues with polymorphonuclear leucocytes, it is possible that the iodide may influence the leucocytes and facilitate the phagocytosis of the injected foreign material producing a heightened inflammatory reaction analogous to the focal reaction not infrequently following the administration of a bacterial vaccine in adequate dosage.

Daily total and differential leucocyte counts of the blood of two persons taking potassium iodide and two others taking potassium bromide until a total of 170 grains has been swallowed, did not show any material influence upon either the total number or variety of leucocytes in the peripheral blood, as compared with a series of preliminary counts before the administration of the drugs. Similar negative results were observed with a series of rabbits receiving the drugs by means of a stomach tube.

The sera of persons and rabbits receiving potassium iodide and potassium bromide appears to have an appreciable increased power for facilitating phagocytosis. As shown in Tables XII and XIII, the sera of persons taking potassium iodide and potassium bromide showed higher phagocytic and opsonic indices for *B. prodigiosis* than the same sera in the preliminary tests before the administration of the drugs; this was particularly apparent with the sera of persons taking

TABLE XII

INFLUENCE OF THE ORAL ADMINISTRATION OF POTASSIUM IODIDE AND POTASSIUM BROMIDE UPON PHAGOCYTOSIS OF *B. PRODIGIOSIS* \*

NAME	DRUG	PHAGOCYTIC INDICES **							
		1st Prelim.	2nd Prelim.	3rd Prelim.	4th Prelim.	After 60 Grains	After 90 Grains	After 140 Grains	After 170 Grains
C. E.	Potass. iodide.....	0.4	0.8	1.0	0.9	1.9	2.6	1.7	1.9
G. H.	Potass. iodide.....	0.4	0.4	1.3	1.6	3.4	1.7	2.0	2.4
A. B.	Potass. bromide.....	0.3	1.0	2.4	1.1	1.9	1.6	0.9	...
S. H.	Potass. bromide.....	0.2	0.6	1.0	0.9	1.9	3.6	1.7	...

\* Tests conducted with the leucocytes of a normal person and not with the patient's own leucocytes.

\*\* Phagocytic indices—the average number of bacilli ingested per leucocyte.

TABLE XIII

INFLUENCE OF THE ORAL ADMINISTRATION OF POTASSIUM IODIDE AND POTASSIUM BROMIDE UPON THE OPSONIC INDEX (*B. PRODIGIOSIS*) \*

NAME	DRUG	AVERAGE PHAGOCYTIC INDEX	OPSONIC INDICES **			
			After 60 Grains	After 90 Grains	After 140 Grains	After 170 Grains
C. E.	Potass. iodide.....	0.7	2.8	3.5	2.4	2.8
G. H.	Potass. iodide.....	0.9	4.0	2.0	2.2	2.7
A. B.	Potass. bromide.....	1.2	1.5	1.3	0	....
S. H.	Potass. bromide.....	0.7	2.8	5.0	2.4	....

\* Tests conducted with the leucocytes of a normal person and not with the patient's own leucocytes.

\*\* Opsonic indices obtained by dividing the patient's phagocytic index by the average phagocytic index obtained with his serum before the administration of the drugs (see Table XII).



TABLE XIV

INFLUENCE OF ORAL ADMINISTRATION OF POTASSIUM IODIDE AND PHAGOCYTOSIS OF B. PRODIGIOSIS \*

RABBIT NO.	PHAGOCYTIC INDICES **								
	1st Prelim.	2nd Prelim.	3rd Prelim.	After 1st Dose	After 2nd Dose	After 3rd Dose	After 4th Dose	After 5th Dose	After 6th Dose
1.....	0.4	1.3	0.4	0.7	0.5	1.5	2.6	5.6	6.7
2.....	0.5	0.4	1.1	0.7	2.5				
3.....	0.3	3.3	1.2	2.8	3.2	3.4	5.3	9.4	10.7
4.....	0.7	1.2	1.9	1.2	1.1	1.6	3.0	4.9	6.2

\* Tests conducted with the leucocytes of a normal rabbit.

TABLE XV

INFLUENCE OF ORAL ADMINISTRATION OF POTASSIUM IODIDE UPON THE OPSONIC INDEX (B. PRODIGIOSIS) \*

RABBIT NO.	AVERAGE PHAGOCYTIC INDEX	OPSONIC INDICES					
		After 1st Dose	After 2nd Dose	After 3rd Dose	After 4th Dose	After 5th Dose	After 6th Dose
1.....	0.7	1.0	0.7	2.0	3.7	8.0	9.5
2.....	0.7	1.0	3.5				
3.....	1.6	1.8	2.0	2.1	3.3	5.8	6.7
4.....	1.3	0.9	0.8	1.2	2.3	3.7	4.8

\* Conducted with the leucocytes of a normal rabbit.

potassium iodide. Even more marked results were observed with the sera of rabbits receiving potassium iodide by means of the stomach tube (Tables XIV and XV).

Phagocytic experiments *in vitro* consisting in exposing emulsions of B. prodigiosis and staphylococcus aureus to varying dilutions of the drugs in normal salt solution under study for an hour at 37° C., followed by the addition of an emulsion of the mixed leucocytes of normal rabbits secured by aleuronant irritation and from the peripheral blood and reincubation for half to an hour, were generally negative. As shown in Table XVI, the 1:4000 dilution of potassium iodide and potassium bromide appeared to raise the phagocytic powers of the leucocytes, while the 1:4000 dilution of potassium chloride exerted a similar, but very slight influence. A similar influence on the part of the drugs upon staphylococcus aureus was not apparent (Table XVII). Occasionally well marked phagocytosis was observed but not higher than shown occasionally in the series of controls.

It would appear, therefore, that the oral administration of potassium iodide and to a lesser extent of potassium bromide, may increase the phagocytic power of the blood serum for B. prodigiosis and this may have some influence upon skin reactions by increasing leucocytic infiltration about the injected mass. Whether these drugs have a similar influence upon other microorganisms or upon the phenomena of infection and resistance in general would appear worthy of further investigation.

TABLE XVI

INFLUENCE OF DRUGS UPON THE PHAGOCYTOSIS OF <i>B. PRODIGIOSA</i> IN VITRO *			
DILUTIONS OF DRUGS **	PHAGOCYTIC INDICES		
	Potass. Iodide	Potass. Bromide	Potass. Chloride
1:200 .....	1.2	1.1	2.2
1:1000 .....	1.1	1.5	2.0
1:4000 .....	2.5	3.5	2.9
1:10,000 .....	0.9	1.4	1.8
Control *** .....	1.1	2.1	1.5

\* The leucocytes of a normal rabbit used.

\*\* In sterile normal salt solution (0.85 per cent).

\*\*\* In sterile normal salt solution (0.85 per cent).

TABLE XVII

INFLUENCE OF DRUGS UPON THE PHAGOCYTOSIS OF <i>STAPHYLOCOCCUS AUREUS</i> IN VITRO *						
DILUTIONS OF DRUG * * *	PHAGOCYTIC INDICES **					
	Potass. Iodide	Sodium Iodide	Potass. Bromide	Sodium Bromide	Potass. Chloride	Ammon. Chloride
1:100 .....	0.9	0.7	0.9	1.0	1.2	0.4
1:500 .....	0.8	0.9	0.5	1.3	0.8	0.5
1:1000 .....	1.1	0.9	0.9	1.2	0.8	0.8
1:5000 .....	0.9	0.5	1.0	1.4	0.8	0.8
1:10,000 .....	0.9	0.5	1.4	1.5	1.3	0.5
1:20,000 .....	1.3	0.8	0.7	0.9	1.2	0.9
1:40,000 .....	0.3	1.2	0.6	1.3	0.8	0.6
1:100,000 .....	1.1	0.5	0.7	1.0	0.8	0.5

\* *Staphylococcus aureus* recently cultivated from the pus of a furuncle. The leucocytes of normal rabbits employed.

\*\* The controls (set up with sterile normal salt solution) showed a spontaneous phagocytosis varying from 2.0 to 0.1 and averaging 0.5 per leucocyte.

\*\*\* In sterile normal salt solution (0.85 per cent).

## SUMMARY.

1. The iodides and particularly potassium iodide were found to influence the luetin and prodigiosis intracutaneous tests to a marked extent. Normal non-syphilitic persons, reacting negatively in the luetin test, may show marked reactions when tested after the oral administration of sixty or more grains of potassium iodide.

2. The bromides of potassium and sodium in the same dosage were found to have a similar but less marked influence.

3. The chlorides of potassium and ammonium in the same dosage were found to influence the prodigiosis reactions but not the luetin reactions except to a very slight extent.

4. The administration of the protiodide of mercury influenced the luetin reaction to some extent.

5. It is probable that the administration of larger doses of these drugs would exert a more marked influence upon skin reactions.

6. Ether and chloroform anesthesia did not appear to influence skin reactions.

7. Substances most likely to excite inflammatory reactions in the skin ap-

pear to be most readily influenced by the iodides and to some extent by bromides; intracutaneous tests with agar-agar, prodigiosin and ordinary luetin were more readily influenced by these drugs than the reactions following the injection of a luetin of washed spirochetes and free of culture medium.

8. Cutaneous tests are not as readily influenced by these drugs as intracutaneous tests.

9. Conjunctival tests among normal rabbits to tuberculin were apparently not influenced.

10. Cutaneous and intracutaneous reactions to tuberculin among persons reacting positively to both, appear to be rendered more extensive by potassium iodide and to a lesser extent by potassium bromide.

11. Anaphylactic reactions to luetin in syphilitic persons appear to be rendered more extensive by potassium iodide and potassium bromide.

12. The oral administration of potassium iodide and to a lesser extent of potassium bromide, increased the phagocytic power of the blood serum for B. prodigiosus; the increased severity of skin reactions in persons taking these drugs may be due to heightened leucocytic infiltration and phagocytosis about the injected material or to an increase of tryptic activity through the saturation of fatty acid radicals according to the hypothesis of Jobling and Petersen.

13. Physicians should very carefully rule out the possible influence of these drugs before conducting skin reactions.

14. It is probable that these drugs have influenced the luetin reactions as clinically applied and have been responsible in part for the divergent results observed and reported.

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# THE DISTRIBUTION OF THE BLOOD SUGAR AS REGARDS CORPUSCLES, PLASMA, AND WHOLE BLOOD IN HEALTH AND DISEASE IN MAN\*

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IT occurred to the writers to determine according to the newer blood chemical methods the exact distribution of the blood sugar in man as regards the corpuscles, plasma, and whole blood. The data which we have accumulated are based upon a number of analyses of blood obtained in the routine way by venipuncture, the blood in all cases being received into potassium oxalate (dried), and the sample taken in the morning before breakfast. The method used was that of Benedict and Lewis, as modified by Myers and Bailey. It is interesting to note that so far as we are able to learn in a search of the literature, no such data have hitherto been obtained by using these methods.

Possibly the most important research along this line is that of Tachau<sup>1</sup> who reported his data on the distribution of blood sugar in blood corpuscles and blood serum. Prior to this publication, Lèpine,<sup>2</sup> Michaelis and Rona,<sup>3</sup> and Hollinger<sup>4</sup> found in the blood serum and corpuscles of man and other animals different amounts of blood sugar. Since then, others have worked along similar lines, notably Rona and Döblin,<sup>5</sup> E. Frank,<sup>6</sup> Lyttkens and Sandgreen,<sup>7</sup> Hoeber,<sup>8</sup> Schirokauer,<sup>9</sup> and others. There are great discrepancies in these results, possibly due to the fact that some work was carried out with human blood and other work with animal blood. Since the appearance of Masing's<sup>10</sup> and Loeb's<sup>11</sup> articles, we know that the sugar content of blood in different animals and in man gives different figures, and also that the variations in the blood sugar in man and in animals quite close to him are different from a metabolic standpoint. It must, however, be noted that the observations of most of these investigators have not been made with respect to an estimation of the normal blood sugar content under ordinary conditions, most of the human data having been based upon a computation after the ingestion of large amounts of carbohydrates and most of the animal data having been procured after the animals were narcotized and tied up for a long time. Michaelis and Rona<sup>12</sup> and E. Frank<sup>13</sup> showed that in the presence of a hyperglycemia due to the ingestion of a large amount of carbohydrates, the blood sugar content of serum is increased more than that of the whole blood or the corpuscles. As for the results in man, Hollinger found that the amount of sugar was the same in whole blood and in plasma. E. Frank, in a number of pathological cases, found more sugar in the serum. Schirokauer, as a rule, in fasting persons, found a higher percentage of blood sugar in plasma than in whole blood or corpuscles. In alimentary hyperglycemia the difference between the whole blood and the plasma was quite marked in a number of cases examined. It was noted that one hour after the ingestion of the dose of carbohydrate that caused the alimentary hyperglycemia, the balance between the two was adjusted so that there was practically no more difference between the blood

\*From the Chemical Laboratory of the Pasteur Institute of St. Louis.

sugar content in carbohydrate-fed persons than that seen in fasting persons. The blood sugar in the corpuscles of alimentary hyperglycemics ran high; in but few cases did the concentration of sugar in the corpuscles remain low; and in one case, when the blood sugar concentration in the whole blood went up, the blood sugar in the corpuscles went down.

As for the different results seen in the case of man, Hollinger found the same amounts of sugar in plasma and in whole blood. E. Frank, in pathological cases, found an increase in the sugar in the serum over the corpuscles, while Schirokauer found great differences between the whole blood and the serum. Tachau worked this matter out on fasting persons. At the same time, Rolly and Oppermann<sup>14</sup> published their figures on fasting persons. Tachau reported both pathological cases and also normal people after carbohydrate ingestion. Owing to the fact that very large quantities of blood were needed with this technic Tachau could not use the same person's blood more than once. His technic was as follows: Blood obtained by venipuncture was received in sodium fluoride, 40 c.c. in quantity, centrifuged in a high power electric centrifuge for fifteen minutes. The blood volume was taken with a Boenning tube. The whole blood and corpuscles were treated according to Schenk's method to precipitate the protein material, and blood sugar estimation made with the Knapp solution as previously reported by Tachau.<sup>15</sup> A point to be taken into consideration in this work is the suggestion made by Lèpine<sup>16</sup> that there is "free" and "bound" sugar in the blood; i.e., that shortly after standing, within fifteen minutes, in fact, some of the "bound" sugar is liberated and becomes "free" sugar, going over from corpuscles to plasma, remaining of course in the plasma. Tachau covered this question in his investigations. A part of the blood was placed directly in water and 2 per cent hydrochloric acid as described in his previous technic; another part was received into the sodium fluoride and allowed to stand one hour in the laboratory. The blood volume was determined by weighing. Table I of Tachau's experiments gives the results on this question.

TABLE I

CASE NO.			PER CENT.	PER CENT AFTER 1 HR.
1.	Potator	(after 100 gm. grape sugar)	0.113	0.111
2.	Liver cirrhosis	" " " "	0.142	0.145
3.	" "	" " " "	0.143	0.142
			0.148	0.148
4.	Erysipelas	" " " "	0.185	0.183
5.	Diabetes (fasting patient)		0.258	0.240
6.	Liver cirrhosis,	(after 100 gm. grape sugar)	0.169	0.177
7.	Gout	" " " "	0.108	0.112
				0.114
8.	Normal,	" " " "	0.086	0.095
9.	Diabetic, fasting	" " " "	0.113	0.125

It will be noted that in the first four cases, the difference between the blood sugar content when first withdrawn and after one hour standing is so slight as to be negligible. In case 5, a fasting diabetic, blood sugar dropped from 0.258 to 0.240 per cent; here perhaps the sodium fluoride caused glycolysis.

In the last four cases, the blood sugar rose after one hour. It can be seen therefore that there is some slight rise, but this is not a constant or important factor.

In Table II of Tachau's figures are seen the results of investigations on fasting people.

TABLE II  
INVESTIGATIONS ON FASTING PERSONS

Case No.	Diagnosis	SUGAR CONTENT							
		Whole Blood %	Plasma %	Difference Between Plasma and Whole Blood %	Quotient Plasma Whole Blood	Blood Volume %	Cor- puscles %	Difference Between Plasma and Cor- puscles %	Quotient Plasma Cor- puscles
1	Pregnancy.....	0.0765 0.0740	0.087	0.012	1.16	36	{ 0.058 0.051	0.029 0.036	1.5 1.7
2	Arteriosclerosis...	0.098							
3	Uremia.....	0.105 0.104	0.110	0.005	1.05	34	0.095	0.015	1.2
4	Nephritis.....	0.111							
5	Diabetes.....	0.129	0.138	0.009	1.07	50	0.120	0.018	1.2
6	" .....	0.150	0.173	0.023	1.15	47	0.125	0.048	1.4
7	" .....	0.153 0.156	0.168	0.014	1.09	....	....	....	....
8	" .....	0.165							
9	" .....	0.183	0.185	0.002	1.01	42	0.181	0.004	1.0
10	" .....	0.243	0.246	0.003	1.01	....	....	....	....
11	" .....	0.258	0.265	0.007	1.03	....	....	....	....
12	" .....	0.301 0.306	0.323	{ 0.022 0.017	1.07 1.06	21	{ 0.219 0.243	0.104 0.080	1.5 1.3

With the exception of case 8, in which all the figures tallied, the sugar concentration was higher in plasma than in whole blood. The average difference was 0.01 per cent. The average quotient was 1.07. As for the volume, the difference between the plasma and the whole blood was greater in ratio to the blood volume; i.e., the greater the difference, the smaller the blood volume. The greatest difference between the plasma and the corpuscles was 0.104 per cent, an average of 0.030 per cent. The quotient average of plasma over corpuscles is 1.3. There seemed, therefore, no more difference between the blood sugar concentration in whole blood, plasma, and corpuscles in individuals with a high or low blood sugar.

Table III by Tachau illustrates the data on blood after the administration of carbohydrates.

The differences between the sugar in the plasma and in the whole blood or corpuscles are greater the higher the hyperglycemia, as opposed to the condition existing in fasting persons. The greatest difference occurred in case 11 0.144 per cent, in a diabetic, the quotient of whole blood over plasma being 1.4. The quotient of plasma over corpuscles was in most cases as much as 2.0. In twelve of these cases where the patients were given carbohydrates followed by a blood test, the quotient of plasma over whole blood was five times higher than in fasting persons (Table II). In the cases where the examinations were

TABLE III  
EXAMINATIONS AFTER INSTITUTION OF CARBOHYDRATES

Case No.	Diagnosis	Remarks	SUGAR CONTENT							
			Whole Blood %	Plasma %	Difference Between Whole Blood and Plasma %	Quotient Plasma	Blood Volume %	Corpuscles %	Difference Between Plasma and Corpuscles %	Quotient Plasma
						Whole Blood				Corpuscles
1	Healthy.....	1 hr. After 100 Gm. Grape Sugar.....	0.090	0.112	0.022	1.25	.....	.....	.....	.....
2	Heart Insufficiency.....	".....	0.093	0.118	0.022	1.29	40	0.053	0.055	2.3
3	Gout.....	".....	0.096	0.120	0.027	1.23	40	0.063	0.063	1.8
4	Nephritis.....	".....	0.112	0.129	0.016	1.15	45	0.127	0.028	1.2
5	Liver Cirrhosis.....	".....	0.114							
6	Uremia.....	".....	0.142	0.155	0.013	1.09	.....	.....	.....	.....
7	Liver Cirrhosis.....	".....	0.143	0.153	0.005	1.03	.....	.....	.....	.....
8	Erysipelas.....	".....	0.148	0.155	0.002	1.08	.....	.....	.....	.....
9	Lead Poisoning.....	".....	0.147	0.160	0.013	1.09	.....	.....	.....	.....
10	Diabetes.....	1 hr. After 50 Gm. White Bread.....	0.169	0.213	0.044	1.26	39	0.100	0.113	2.1
11	".....	".....	0.182	0.207	0.024	1.13	47	0.185	0.052	1.3
12	".....	".....	0.185							
13	Healthy.....	1 hr. After 100 Gm. Grape Sugar.....	0.213	0.237	0.024	1.11	47	0.185	0.052	1.3
14	Drinker.....	1 hr. After 100 Gm. White Bread.....	0.221	0.231	0.010	1.05	50	0.212	0.019	1.1
15	".....	".....	0.334	0.480	0.146	1.44	.....	.....	.....	.....
16	".....	".....	0.361	0.387	0.026	1.07	41.5	0.325	0.062	1.2
17	Healthy.....	1 1/2 hrs. After 100 Gm. Grape Sugar.....	0.093	0.075	0.018	0.80	47	.....	.....	.....
18	Drinker.....	1 3/4 hr. After 100 Gm. Grape Sugar.....	0.111	0.126	0.014	1.13	.....	.....	.....	.....
19	Arteriosclerosis.....	2 hrs. After 100 Gm. Grape Sugar.....	0.113							
20	".....	".....	0.056	0.058	0.002	1.03	46	0.054	0.004	1.07
21	".....	".....	0.092	0.086	0.006	0.94	40	0.100	0.014	0.86
22	Carcinoma Liver.....	".....	0.111	0.129	0.018	1.16	37	0.081	0.048	1.6
23	Liver Cirrhosis.....	".....	0.142	0.188	0.043	1.30	36	0.051	0.142	3.8
24	Diabetes.....	".....	0.145	0.193	0.051	1.36		0.070	0.118	2.7
25	Acromegalia.....	".....	0.180	0.225	0.045	1.25	37	0.103	0.122	2.2
26	Diabetes.....	".....	0.206	0.240	0.034	1.17	30	0.127	0.113	1.9
27	".....	".....	0.295	0.312	0.017	1.05	47	0.277	0.035	1.1
28	".....	2 1/4 hrs. After 50 Gm. White Bread.....	0.126	0.126	0	1.00	31	0.126	0	1.0
29	".....	2 1/4 hrs. After 100 Gm. White Bread.....	0.325	0.344	0.019	1.06	41.5	0.300	0.044	1.1
30	".....	3 hrs. After 150 Gm. White Bread.....	0.428	0.386	0.042	0.90	45	0.480	0.094	0.8
31	".....	4 hrs. After 50 Gm. White Bread.....	0.234	0.228	0.006	1.00	42	0.243	0.015	0.9

made one hour after carbohydrates were ingested, the whole blood was higher in sugar than was the plasma. In cases 13 and 24 the differences were so great that they could not possibly be due to errors in technic or calculation. In one case the sugar concentration in whole blood went up and that of the corpuscles

diminished, due to the fact that the corpuscles must have yielded up some of their sugar. This phenomenon was first noted by Rona and Takahashi<sup>17</sup> and E. Frank and Bretschneider.<sup>18</sup> Tachau also claims that the increase in sugar concentration in the corpuscles, observed in alimentary hyperglycemia, was due to the relative permeability-increase in vitro in human corpuscles for grape sugar, as suggested by Rona and Doblin, Hoeber and Masing.<sup>10</sup> We can think of it in this way: when the alimentary hyperglycemia begins and sugar is thrown in increased quantity into the circulation, it is first dissolved in plasma and penetrates the corpuscles secondarily. As the hyperglycemia declines, the sugar content of the plasma goes down and the corpuscles then throw their sugar in excess into the plasma. Tachau attempts to explain by this line of reasoning why it is that in the presence of a declining hyperglycemia of alimentary origin, the serum loses its sugar, and strange to say, the corpuscles then hold more sugar than the plasma. Perhaps this is due to a sudden liberation of sugar from the blood stream. In this connection the work of E. Masing<sup>19</sup> bears strongly on this point: he showed by exhaustive experiments that the addition of sugar to a quantity of blood in vitro is followed by the taking up of the sugar by the corpuscles first; then later the corpuscles give up this sugar excess and in the final analysis sugar in larger quantities is found in the plasma. This is in confirmation of the work of Rona<sup>20</sup> and the prior publication of Masing.<sup>21</sup> Masing further showed that the addition of sugar to blood was followed by the

TABLE IV  
INVESTIGATIONS ON THE SAME PERSONS AND EXPERIMENTAL ANIMALS

Case No.	Diagnosis	Remarks	SUGAR CONTENT					Quotient Plasma
			Blood Volume C <sub>c</sub>	Whole Blood C <sub>c</sub>	Plasma C <sub>c</sub>	Corpuscles C <sub>c</sub>	Difference Between Plasma and Corpuscles C <sub>c</sub>	
1	Nephritis.....	Fasting 1 hr. After 100 Gm. Grape Sugar..	45	{ 0.111 0.142	{ 0.126 0.155	{ 0.093 0.125	{ 0.033 0.033	1.3
2	Diabetes.....	Fasting 1 hr. After 50 Gm. White Bread..	50	{ 0.129 0.221	{ 0.138 0.231	{ 0.120 0.212	{ 0.018 0.019	1.2 1.1
3	" .....	" .....	.....	{ 0.243 0.334	{ 0.246 0.480	.....	.....	.....
4	" .....	Fasting 2 hrs. After 100 Gm. Grape Sugar..	47	{ 0.150 0.295	{ 0.173 0.312	{ 0.125 0.277	{ 0.048 0.035	1.4 1.1
5	" .....	Fasting 1 hr. After 100 Gm. White Bread..	41.5	{ 0.361 0.325	{ 0.387 0.344	{ 0.325 0.300	{ 0.062 0.044	1.2 1.1
6	Dog 1, Police Dog.....	Fasting ¾ hr. After 100 Gm. Grape Sugar..	43	{ 0.081 0.213	{ 0.090 0.264	{ 0.070 0.146	{ 0.020 0.118	1.3 1.8
7	Dog 2, Bull Dog	Fasting 1 hr. After 80 Gm. Grape Sugar..	36	{ 0.081 0.150	{ 0.087 0.159	{ 0.069 0.133	{ 0.018 0.026	1.3 1.2
8	" .....	Fasting 1¼ hrs. After 120 Gm. Grape Sugar..	36	{ 0.082 0.223	{ 0.087 0.174	{ 0.072 0.311	{ 0.010 0.137	1.1 0.6



slow entrance of sugar into the corpuscles at zero Centigrade, faster at 25° C., and that this entrance was hindered markedly by high temperatures. Masing also showed that treatment of corpuscles with formalin enhanced their permeability for sugar.

The fourth table of Tachau's data on the same persons and on experimental animals, eight cases in all, showed a great difference between the sugar content of plasma and corpuscles in case 4, a diabetic, blood taken two hours after 100 grams of grape sugar were administered. The difference was 0.048 per cent. The greatest difference was in case 5, 0.062 per cent, a diabetic, fasting one hour after 100 grams of white bread were ingested. It is significant to note that the three dogs examined, cases 6, 7, and 8, showed about the same percentage of blood sugar in their whole blood: viz., 0.081, 0.081, and 0.082 per cent, respectively. The least difference found between plasma and corpuscles was in case 8, 0.010 per cent, and the greatest in case 5, 0.062 per cent.

Our figures are based upon a comparison of the blood sugar content of 24 cases, using the latest method, that of Benedict and Lewis<sup>22</sup> modified by Myers

TABLE V  
ANALYSIS OF WHOLE BLOOD, PLASMA AND CELLS

NO.	NAME	SEX*	DATE	WHOLE BLOOD	PLASMA	CELLS	REMARKS
1	W.M....	♂	8/8	0.135	0.135	0.135	Patient normal. Blood taken after breakfast.
2	F.B.....	♂	8/15	0.132	0.129	0.132	Patient normal. Blood taken after breakfast.
3	Dr.H....	♂	8/16	0.204	0.204	0.200	Patient diabetic.
4	M.H....	♂	8/18	0.156	0.153	0.156	Patient epileptic.
5	Dr.H....	♂	8/19	0.165	0.162	0.165	Patient fasting six days.
6	C.B.....	♂	8/22	0.159	0.155	0.159	Patient syphilitic. Blood taken after dinner.
7	F.H....	♂	8/22	0.140	0.138	0.140	Patient syphilitic. Blood taken before breakfast.
8	E.B.....	♂	8/23	0.300	0.225	0.240	Case of a boy of 12 years who was dying at the time blood was taken (DIABETIC COMA).
9	B.E.S....	♂	8/31	0.132	0.129	0.132	Blood taken after injection of salvarsan.
10	J.W....	♀	9/13	0.200	0.196	0.196	Patient diabetic.
11	Dr. H....	♀	9/16	0.225	0.225	0.225	See case No. 3 and 5.
12	9697....	♂	9/18	0.135	0.135	0.132	Patient syphilitic. Wassermann + + + +
13	G.M....	♂	9/18	0.123	0.123	0.123	Patient syphilitic.
14	M.R....	♀	9/20	0.345	0.340	0.340	Patient diabetic.
15	E.G....	♂	9/21	0.144	0.144	0.144	
16	J.R....	♀	10/3	0.120	0.120	0.120	Patient on "ALLEN TREATMENT" since 9/27.
17	J.R....	♀	10/19	0.129	0.129	0.129	See Case No. 16.
18	M.M....	♀	10/20	0.090	0.090	0.090	Patient pregnant and has only one kidney.
19	F.S.....	♂	11/10	0.102	0.099	0.102	Patient normal. Blood taken after breakfast.
20	M.S....	♀	11/20	0.138	0.138	0.138	Patient on "ALLEN TREATMENT" since 11/17.
21	G.D....	♀	11/21	0.102	0.102	0.099	Patient syphilitic and has a trace of sugar in urine.
22	P.R....	♂	11/21	0.087	0.084	0.087	Blood taken one hour after injection of salvarsan.
23	M.G....	♂	11/25	0.210	0.207	0.210	Patient syphilitic.
24	B.P....	♂	11/25	0.120	0.120	0.117	Patient diabetic.

\* ♂Signifies Male.  
♀Signifies Female.

and Bailey.<sup>23</sup> The blood was diluted one to five with distilled water, immediately after withdrawal, precipitated with picric acid, mixed with a stirring rod, and allowed to stand with occasional stirring. The tube is now centrifuged for a few minutes and the supernatant fluid filtered into a dry test tube through a small thick piece of filter paper. Three c.c. of the filtrate are pipetted into a specially graduated test tube, 1 c.c. of 20 per cent sodium carbonate added, and the solution heated for fifteen minutes for the development of color. The solution is allowed to cool, made to volume with water, 10, 15, or 20 c.c., dependent upon depth of color, mixed and compared in the Hellige colorimeter with the wedge of standard picramic acid. In these determinations, of course, we worked with another part of the same blood, which was strongly centrifuged beforehand, in that way separately gathering the plasma and the cells. The plasma and the cells in turn were handled in the same way as was the whole blood.

Table V shows the results of our own investigations.

From a study of these twenty-four examinations it can be readily seen that the quantity of sugar in the whole blood, in the plasma, and in the corpuscles in nearly all cases is the same. Our cases were normal individuals, syphilitics, diabetics before and after undergoing "Allen" treatment, and one epileptic. Our figures agree rather closely with those of Tachau already cited at length. In but one case, No. 8, did we see a wide variation from this agreement: here we had 0.30 per cent in the whole blood, 0.225 per cent in the plasma and 0.24 per cent in the cells. This was a very interesting case of a boy of twelve years who died within twenty-four hours after admission into the City Hospital of what was judged to be diabetic coma. The thought suggested itself that in the terminal stages of life, in diabetes, there is perhaps a variation in the sugar content of the various parts of the blood, but as yet we have had no opportunity in diabetic coma cases to verify this observation.

#### CONCLUSION.

Using the latest methods of sugar analysis in blood, namely, that of Lewis and Benedict as modified by Myers and Bailey, we find that the amount of sugar is practically the same in the whole blood, plasma, and cells. This is in the main in perfect agreement with the work of Tachau who used the older technic of sugar estimation. This seems to disprove the theoretical views of some of the older physiologists who held that a part of the sugar in the blood was in a state of loose combination with some other substance. This obsolete idea has, of course, already been considerably shaken by the work of Rona and Michaelis<sup>24</sup> who showed that blood sugar is in a state of solution; they showed that when diluted blood is shaken with certain colloids, such as ferric chloride or kaolin, the proteins form a colloidal combination, and are absorbed. They can then be quantitatively precipitated by the addition of a trace of electrolyte, but no trace of sugar is removed from the solution by this treatment. If the sugar were united with the proteins it would be carried down with them, and as the reagents used cannot have any disruptive effect, it is not possible for the sugar to exist in combination with the proteins. As Cammidge<sup>25</sup> states, too, another piece of evidence in support of the free state of dextrose in the blood is

furnished by the observation that, whereas charcoal absorbs both sugar and protein when shaken with a solution containing these two substances, yet it absorbs the protein, but not the dextrose, when acetone is present. The acetone being more absorbable than the dextrose, prevents the latter being taken up by the charcoal. Further evidence is also furnished by the results of dialysis experiments.

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# LABORATORY METHODS

## A NEW KIND OF LONG PAPER KYMOGRAPH\*

By D. E. JACKSON, PH.D., M.D., ST. LOUIS, MO.

ALMOST the entire framework of the long paper kymograph† shown in the accompanying illustration is made of regular standard gas pipe and fittings. Three sizes of pipe (and fittings) are used in the construction of the base or supporting table, viz., 1, 1¼ and 1½ inches. The main horizontal shaft to which the drums are attached is constructed of inch piping except in the middle portion where 1¼ inch sized fittings are used in order to make a stronger hinge. The small frames to hold the drums, etc., are of smaller piping (¾ or 1½ inch).

When the "lock pin" is pulled out (upwards) the entire drum shaft and drums can be turned over (backwards in the illustration) downwards until the center of the horizontal shaft rests on the "smoking rest arm." The drums are then horizontal and in the correct position for smoking the paper which is pasted together at the ends and passed like a belt around the drums. The left hand drum is free to turn. The belt is dropped off one of the pulleys at the right hand drum and then the "crank for smoking" is used to turn the right hand drum rapidly to smoke the paper.

The paper passes along just below (outside) the smoking plate. This plate is made of cast iron and is 12 by 6 by ¼ inches in dimensions. The smoking flame is held just below the smoking plate which keeps the paper from getting too hot.

The left hand drum is held in a frame which slides on two parallel (1 inch) pipes which extend from the smoking plate to the left hand end of the horizontal shaft. The fittings marked "drum slide" and "B" are bored out on the inside to slide over the parallel pieces of piping.

The drums may be made of sheet brass rolled to fit the heads (ends) which can either be made of cast brass or simply cut (turned) from a sheet of brass (about ⅛ inch thick, or galvanized sheet iron 1/16 inch thick). It is much cheaper, and perhaps better in every way, to make the cylindrical portions of the drums of galvanized sheet iron (1/16 inch thick). These pieces can easily be trimmed to the proper size and rolled into perfect cylinders at a very nominal cost by any sheet metal company. The parts of the drums are easily soldered together.

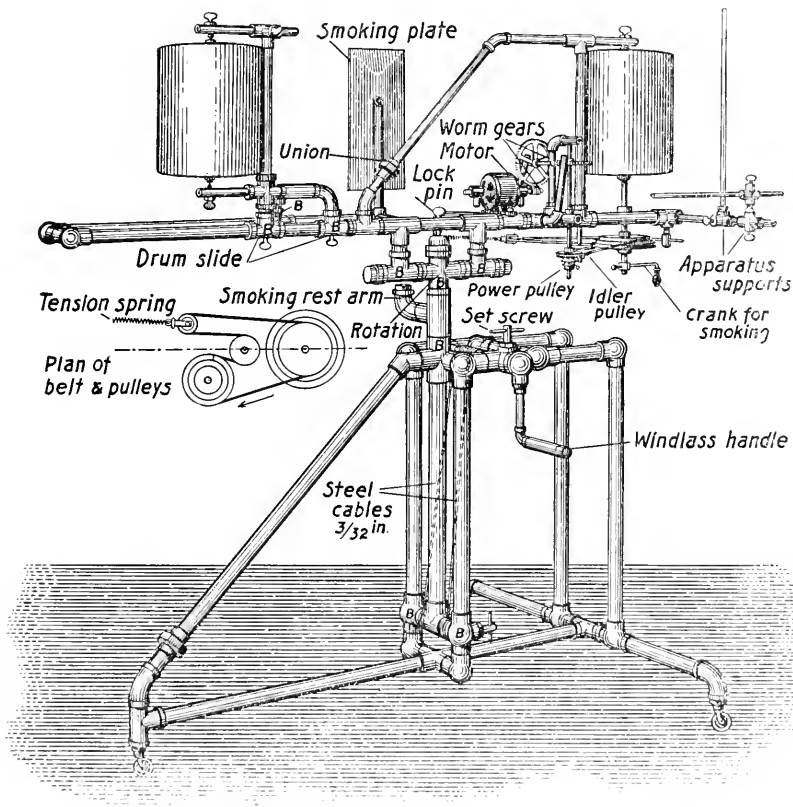
A small motor (costing \$3.50 and purchased, second hand, of the Gregory Electric Company, 16th and Lincoln Streets, Chicago) has been used to turn the drums. The motor is 1/20 horse power and revolves 1500 times per minute (Robbins and Myers).

It may perhaps be advisable to use a slightly stronger motor (1/16 horse

\*From the Department of Pharmacology of Washington University Medical School, St. Louis.

†The kymograph here described was demonstrated in New York on December 29, 1916, before the Federation of American Societies for Experimental Biology.

power) if some difficulty is experienced in getting gear wheels that are entirely satisfactory. I have used two sets of worm gears as indicated in the picture. The large gear wheel may have from 120 to 140 teeth (motor 1500 R.P.M.) and the smaller wheel (horizontal) has 84 teeth. But for motors of different speeds these gears must be bought to give the desired drum speeds. A large number of different speeds may be secured for the drum by having several different sized pulleys (which are interchangeable) to place on the lower end of the power spindle (power pulley) and on the lower end of the drum spindle. These spindles are all made of  $\frac{1}{2}$  inch mandrel iron (*shafting*). The pulleys



Motor driven long paper kymograph. (For description see text.)

are attached by set screws and can be readily removed or interchanged. The round leather belt is kept taut by a tension spring (see plan of belt and pulleys).

Adjustable and interchangeable apparatus supports are attached to the right hand end of the horizontal shaft.

The main horizontal shaft and the drums are supported by a sliding upright shaft of  $1\frac{1}{2}$  inch piping. At the lower end of this shaft is placed a tee which connects with two more side-outlet tees which are bored out (marked B) to slide on the two outer upright bars. A set screw is shown in one of these side-outlet tees. The set screw is used to fasten the sliding tee at any desired height.

The central supporting shaft also slides through another side-outlet tee at the upper part of the table. The portions of the hinge marked "B" are also bored out to rotate on the short pieces of piping which pass through the fittings.

The drums are raised or lowered by means of a windlass and a steel cable (3 32 inch in diameter). The cable is fastened to the lower end of the supporting shaft and passes upward (double) to the windlass which can be locked by a set screw. The fittings through which the roller of the windlass passes are bored out to permit rotation of the roller (1 1/4 inch piping) as the crank is turned.

The main horizontal drum shaft and drums can be rotated in a horizontal plane around the central axis. This is accomplished by the use of a cross, the perpendicular openings of which are bored out to turn on the upper end of the main supporting shaft (at the point marked "rotation" in the illustration). On the extreme upper end of the main supporting shaft is placed a cap. Through this cap the lock pin passes down from the two holes in the horizontal shaft above. If the lock pin is pulled out and the cap screwed off, then the entire upper part of the kymograph can be lifted off from the base. This is a matter of great convenience for shipping, etc.

Castors (as shown in the picture) are not necessary and are perhaps best avoided for ordinary routine purposes.

The two drums here shown are 10 inches high and 8 inches in diameter. For those who so prefer it is a very easy matter to attach a small extra drum (or cylinder) to the frame which holds the right hand drum. This extra drum can be used to furnish a *flat* recording surface for the writing points of the tambours, etc. This is accomplished by passing the belt of drum paper around all three drums.

"Ball" pattern fittings (railing fittings) have been used in a number of places in the construction of the framework because they are somewhat neater in appearance than the ordinary styles of fittings.

The kymograph here shown and described should be considered merely as typical of the many forms and sizes of kymographs which can easily and cheaply be constructed of the materials here indicated. For the particular kymograph illustrated here the cost of materials, including the labor of a steam and gas fitting company, which made up the base and the main portions of the upper part of the framework, and the labor of another firm which rolled and soldered the brass cylinders, was a little more than \$40.00. But this price is higher than it should probably have been because a little "experimenting" had to be done in the work of construction. The attachment of the motor, gears and pulleys, etc., represented a further small expense.

In many schools it should easily be possible to have a sufficient supply of these long paper kymographs constructed either in the departmental shops or in other departments in the school in which mechanical work is being taught. In these cases, if a plumbing company can be hired to make the framework, and a sheet metal company to roll the drums, the total cost of construction should not exceed \$75.00 and might be considerably less. The belt pulleys should be made of cheap cast iron. The set screws can be obtained from the stock of any large hardware store. The gear wheels and worms cost about

\$3.30. The motor must be bought to fit the current available in the laboratory, and the switch used for starting or stopping the motor can be placed on an extension cord, thus allowing the operator to control the drum from any place about the room. A spinning device for the right hand drum can easily be attached to the drum spindle.

## THE CONSTRUCTION OF CLINICAL LABORATORY EQUIPMENT

BY MILES J. BREUER, M.D., LINCOLN, NEB.

THERE are many practitioners who would be desirous of availing themselves of the modern clinical laboratory methods, were they not deterred therefrom by the high cost of equipping an efficient laboratory. If a practitioner is to have his own laboratory, he must have the various time- and labor-saving devices; otherwise, the routine performance of many laboratory procedures would be out of the question for a man who has patients to take care of. Yet, the cost of this apparatus is so high as to make it out of the reach of many. The writer wishes to show how some of these pieces of apparatus may be constructed by the physician himself.

These remarks will appeal especially to the young man in practice, who has been trained in the modern scientific methods, and is eager to apply them for the benefit of his patients, yet finds in the course of the doctor's rather difficult beginning-period, that he cannot afford to purchase the needed apparatus. He is the very one who will have the time that it takes to construct the apparatus.

In fitting up the laboratory, a good microscope is the biggest item of expense, and of course, the purchase of this is unavoidable. The various small pieces of glassware are not a great item of cost, with the exception of the hemocytometer. The first thing on which the physician can increase his efficiency without great expense, is the centrifuge. A hand centrifuge can be purchased at a moderate price, but this is useless for anything except urinalysis, and very slow and laborious for this purpose. With an electric centrifuge, one can be attending to other tasks while the specimen is whirling, and cut down the time spent in the laboratory, by a great deal. A good electric centrifuge is rather expensive. The writer's first electric centrifuge was a discarded electric-fan motor, on which were fastened the arms and shields from a hand centrifuge, and this performed excellent service for a long time. Small electric motors to run on the 110-volt current can always be procured at second-hand; in most large cities there are regular exchanges handling them, and a very few dollars paid for one of these, will be many times repaid by the added convenience and efficiency for the man who feels that the price of an electric centrifuge is too much for him.

The next piece of apparatus of importance is an incubator. The lack of an incubator is the only thing that stands in the way of many men doing for them-

selves certain very useful things, such as diphtheria cultures and autogenous vaccines. The making of an electric incubator and thermostat is very simple; and once adjusted, the apparatus requires no care, except to switch it on and off when required, like an electric light.

Our incubator was made by starting with a cubical frame, a foot on each edge, constructed of 1-inch sticks;  $\frac{1}{4}$ -inch cypress boards were used to cover this, both inside and out, thus making a double-walled box with a 1-inch space

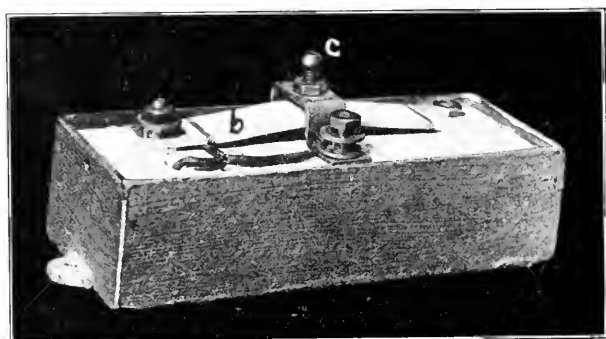


Fig. 1.—Window flasher, original form.

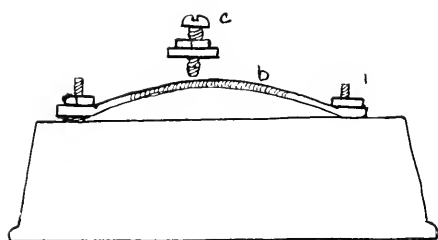


Fig. 2A.—Original form of "flasher."

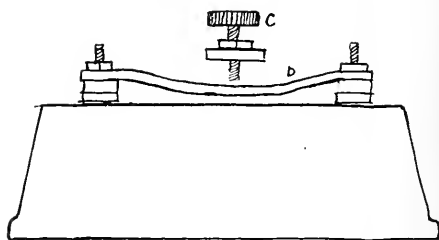


Fig. 2B.—"Flasher" altered for thermostat.

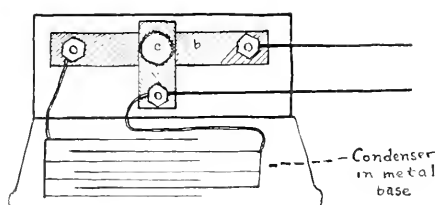


Fig. 2C.—Connections of thermostat.

between the walls. In this space were wrapped several thicknesses of newspaper (newspaper being one of the best heat-insulators we have). An inner glass door was provided and an outer wooden one. The details of construction are not given, as they are trivial, and may be left to anyone who makes such a box. The heat is furnished by a carbon-filament incandescent lamp. The carbon filament produces much more heat than the tungsten. The heating is rendered much more even if an ether can with the top and bottom melted off, mounted on three wire legs, is placed around the incandescent globe. The globe



may be dipped in shellac colored red with carmine, eosin, or safranin (fuchsin will not do, as it changes color) to protect the cultures from the bactericidal effect of white light. A snap switch is mounted on the outside for turning the heat on and off. Where the electric wires pass through the wall of the incubator, the holes should be protected by porcelain bushing. A thermometer is suspended through a hole in the top, and rendered tight by putting it through a cork stopper.

The thermostat was made from one of the "window flashers" which is shown in Fig. 1, and which can be purchased at any electrical supply store. Such a piece of apparatus would not be difficult to make, but this was chosen for

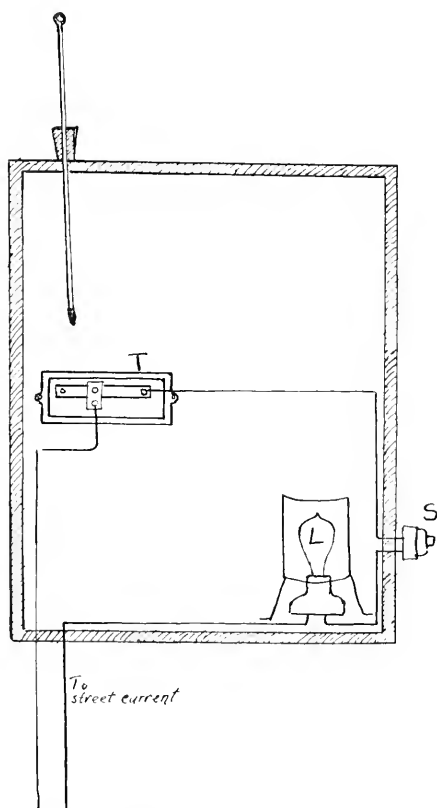


Fig. 3.—Diagram of incubator connections.

the reason that it met with the underwriters' approval, which a "homemade" piece of apparatus would not, even though it might be even more safe and efficient. There is a great difference in price between this apparatus and a regular electric thermostat for incubators.

The curved metal bar *b*, on the porcelain base, is wrapped with fine insulated wire. This is not required for our purpose, and should be disconnected and cut off. The bar is then reversed, so that the convexity is downwards instead of upwards. In order to effect this, it is necessary to block up the ends by means of two screw-nuts. Bolts and nuts should fit very tight; if they do not, larger ones should be put in, as everything depends on the ends of the bar being

fixed absolutely rigid. The expansion of the bar increases the degree of convexity, depresses the middle portion and breaks the contact when the proper degree of temperature has been reached. The platinum contact may be transferred from one side of the bar to the other, by a jeweler if one cannot do the work oneself, or the bar may be simply bent in the other direction. For a long time we used our apparatus with the original contact-screw *c*, but later replaced it with one having a large milled head and a finer thread, making adjustments finer and more accurate. The wires that extend into the porcelain block connect

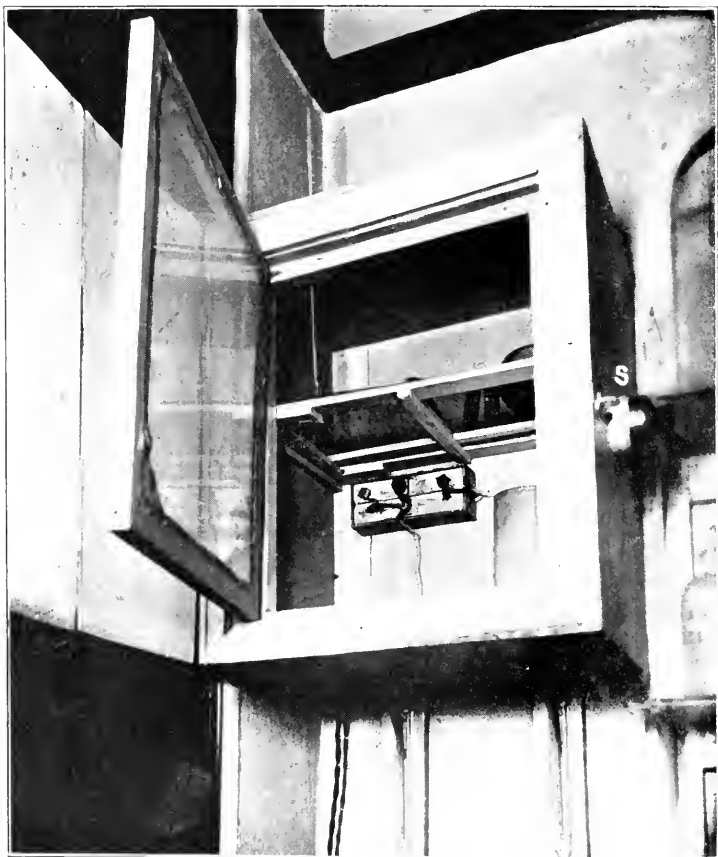


Fig. 4.—Incubator.

with the condenser, which prevents excessive sparking, and which should be connected as shown in *C*, Fig. 2. The connections for the incubator are shown in Fig. 3.

To adjust the thermostat, the contact-screw *c* is set down far enough to maintain a contact. The thermometer is watched through the glass door, keeping the outside door closed between observations. When the temperature has reached 37.5 degrees C, the door should be opened and the contact-screw quickly turned to the left until the light goes out and no further. The setting of the screw may have to be repeated to make the adjustment accurate; the idea is to have

the circuit broken at the exact instant that the temperature reaches 37.5 degrees C. If the box has been tightly made, if the bar on the thermostat is rigidly mounted, and the set-screw *c* is steady, this incubator will hold its temperature accurately and without attention.

The next two pieces of apparatus described are not strictly necessary in the practitioner's laboratory, but they effect such a saving of time that the trouble taken in their construction is many times repaid. In the performance of the complement-fixation test, it is the inactivation and the incubation which the man in practice finds most irksome; watching a water-bath with a thermometer in one hand and a Bunsen-burner in the other, consumes so much time that he is

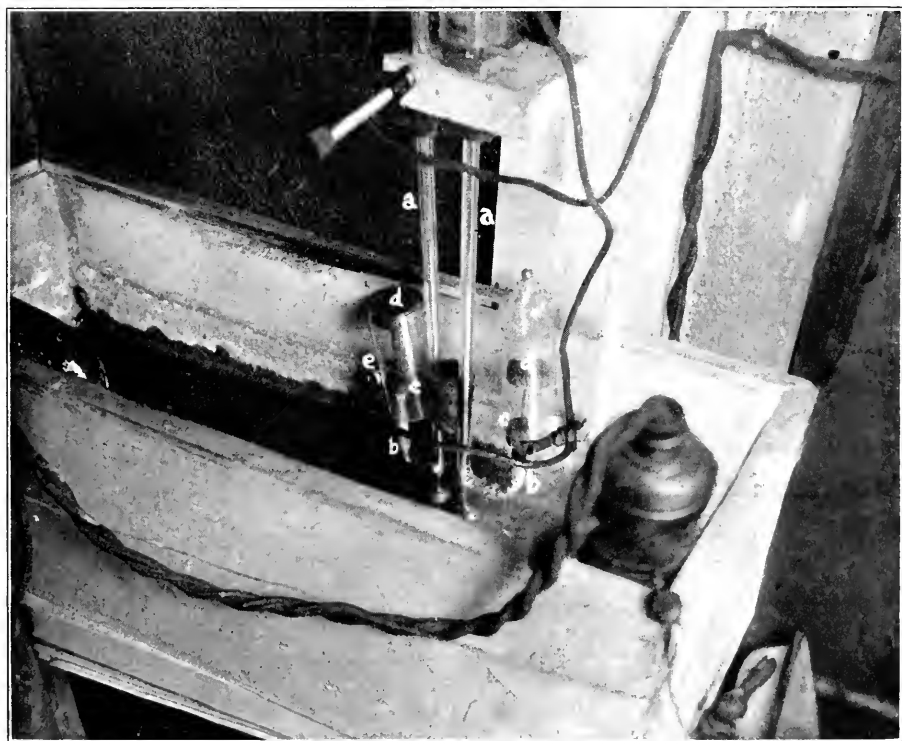


Fig. 5.—Water-bath.

unwilling to do the work. By means of an automatic water-bath, these portions of the process may be left to take care of themselves; and if there is a good centrifuge, there is no excuse for anyone's not performing his own complement-fixation tests. The same applies to the shaking-machine. Our shaking-machine, though originally made to aid in shaking up autogenous vaccines, now finds numerous other applications, as in shaking blood-counting pipettes, sputum for concentration methods of search for tuberculosis bacilli, urine-Obermayer mixtures for the indican test; in fact, every day we find a new use for it. To a busy man, the shaking-machine and the automatic water-bath may mean the whole difference between being able to make his own autogenous vaccines, and not being able to do so; with these two pieces of apparatus, all that will be required of

him is the transplantation of the cultures, and timing when in the various apparatus.

Our water-bath was made from a galvanized-iron box, 6x6x14 inches (constructed for us by a tinner), with a pet-cock in one end by means of which the water can be drained out when desired. A wooden box was built around this, with newspapers between. The heat to the water is supplied by two carbon-filament incandescent globes. Some of the immersion water heaters on the market were tried, but were found to remain hot too long after the current was turned off, causing the temperature to mount too high. (We are contemplating the construction of a special heating unit for this purpose.) The electric globes are sufficient for this purpose, if the glass part is kept submerged in the water by means of wire racks.

The thermostat is the most difficult portion of the apparatus to make. If one is not versed in glass working, the glass portion may be procured to order from any house which handles glass apparatus; and the cost ought to be small, as the apparatus is very simple. However, as it involves only the simplest elements of glass working, there are many who will be able to make the apparatus themselves. The writer is of the opinion that anyone who attempts to work in a laboratory ought to be acquainted with at least the rudiments of glass working and blowing. There is nothing difficult about it. A small gasoline torch will supply sufficient heat; or blasts may be purchased for a small sum. Fig. 9 shows a blast which the writer constructed himself from an old Bunsen burner, by soldering a small brass tube inside, discarding the base, soldering up the side openings tight, and hammering the end (B) until shaped as shown. Gas is connected at G, and compressed air (with which our building is equipped) at A. (The pump-tanks used in nose and throat work for atomization will furnish all the pressure necessary). It will certainly be worth any laboratory worker's time to spend his spare moments for a couple of weeks experimenting with glass tubing, in the ability gained to fashion various minor pieces of apparatus for himself.

The thermostat (A and B, Fig. 6) consists of a small bore tube, of about 1 mm. internal diameter and 20 cm. long; the actual diameter being immaterial, if it is small enough to afford considerable rise and fall of the mercury on change of temperature. A bulb is blown on the end of it, keeping in mind that it must be constantly rotated, in the flame and out of it; and one must blow only a little at a time, and do so repeatedly, in order that the walls of the bulb may become thick enough to stand the weight of the mercury. One or two cm. above the bulb, a small opening is blown in the side of the tube, by making the flame very small and pointed, and carefully blowing out the softened glass. Another tube (which must be prepared beforehand) has the edge on one end thinned by blowing a bulb so rapidly that it bursts, and breaking or filing off any flanges that project more widely than the outer diameter of the tube. This thinned end is heated to redness at the same time that the long tube with the side-hole is kept in the flame; the two pressed together; and the thick portion where the joint is, blown gently outward to make the welding

thorough and decrease the thickness. The side arm can then be bent upward at a right angle. Bending is best done in the luminous gas flame.

The upper contact is made by inserting into the long upper capillary portion of the instrument, a wire tipped with platinum (a jeweler can solder the platinum). The side arm needs provision for adjusting the height of the mercury. A large nut *c* is hollowed out with a drill on one side so that the tube *b* fits into it. It is cemented to the tube with litharge and glycerin mixed so as to form a

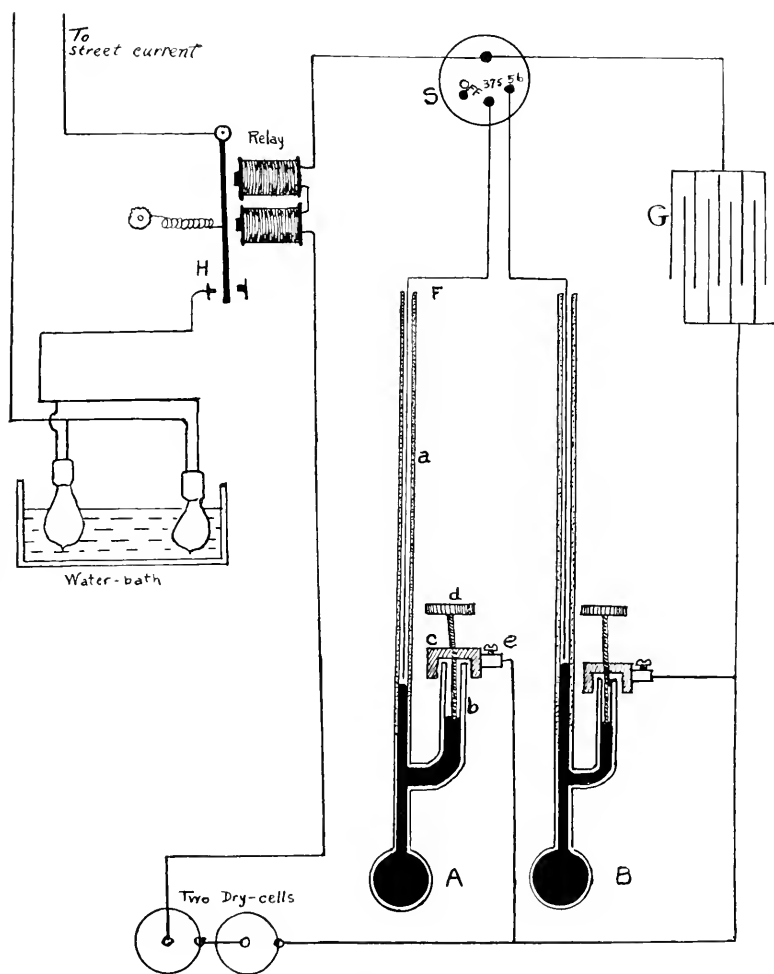


Fig. 6.

thick paste, and allowed to harden in a warm place for several days. A thumb-screw *d* to fit the thread of *c* and to enter the bore of the side arm *b* dips into the mercury and changes its height. A connector *e* for wire, is soldered to the nut.

Two such thermostats were made, in order that the two temperatures commonly used, 37.5 and 56 degrees centigrade could be available without readjusting the instrument each time; and by means of a three-point switch at S, the desired one can readily be thrown into connection. To prevent excessive spark-

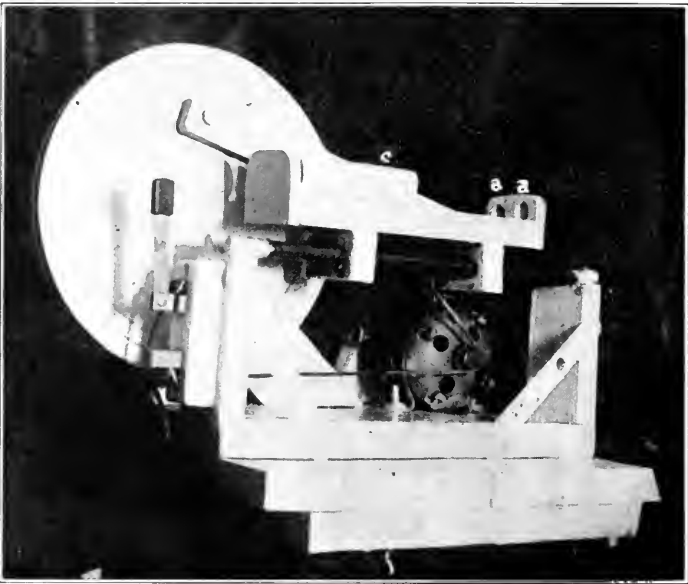
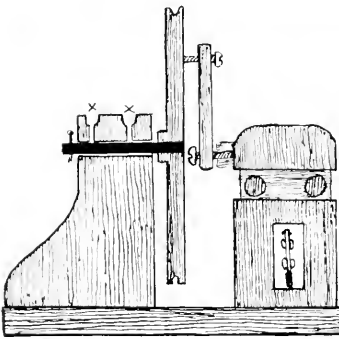
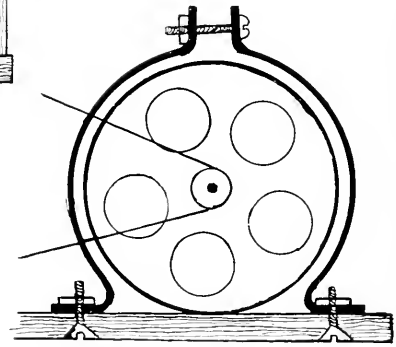


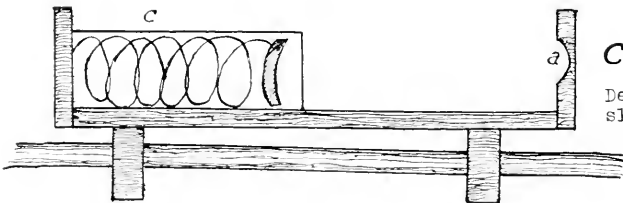
Fig. 7.—Shaking machine.



B  
End projection of  
shaking-machine



A Method of mounting motor.



C  
Details of  
sliding-bed.

Fig. 8.

ing on the mercury surface, due to the coils of the relay, a condensor *G* is thrown between the circuit-breaking terminals of the thermostat, as shown in the illustration. This was made from fifty sheets of tinfoil 3 inches square, interleaved with paraffined paper 5 inches square; the 1st, 3rd, and odd numbered sheets projecting over one end to connect together to one terminal, and the 2nd, 4th, and even numbered sheets over the other end for the other terminal. Care must be taken that there is no short circuit between the terminal (a current should not pass through the condensor).

A 20-ohm telegraphic relay operates the heating circuit. On the relay it will be necessary to reverse the position of the two contact screws which stop the motion of the armature on each side; the one with the hard-rubber tip goes on the same side with the coils, so that in a position of rest, the relay circuit is closed at *H*, and opens when a current is sent through the magnets. The wires from the thermostat circuit should be connected to the posts where the words "20-ohms" are found; the other two are for the heating circuit.

To adjust the thermostat, with the switch at *S* on the proper point, screw up the thumbscrew *d*, so as to break the contact of the mercury with the wire in the tube *a*, thus opening the heating circuit. Allow the temperature to reach the

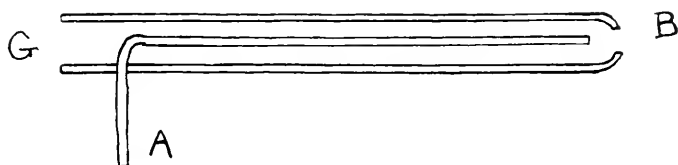


Fig. 9.

desired point (as 37.5 degrees C); then quickly screw down the thumbscrew, making the contact in the tube *a* between mercury and platinum-tipped wire. This closes the current through the relay, causing the latter to break the heating circuit, which remains open until the temperature of the water drops low enough so that the fall of the mercury breaks the thermostat circuit. This may be repeated for finer adjustment. It is advisable to have a cover fitted over the water-bath with openings for the thermostat tubes, as the bath keeps its temperature better and is more economical of current.

The construction of the shaking machine will be clear enough from the illustration, and will require but little explanation. The motor is from a barber's electrical hand-fan, which was obtained at second-hand. It is mounted on the baseboard by means of two iron straps, bolted to the baseboard and extending around the body of the motor, drawn together at the top by means of a stove bolt. The large pulley is of wood, 9 inches in diameter, with a rigidly fastened metal axle. This was procured from a planing mill. The small one is of brass and was procured at a machine shop. The belt is a leather string from a fly-net such as are used on harness in summer. The bearing of the axle of the large pulley is of oak and is three inches long in order to render the pulley steady. From above are bored two holes, widened at the top, to provide for lubrication, as in *B*, *x*, Fig. 8. The same provision is made on the crank-shaft, which is

pivoted to the large pulley and the sliding-bed. The crank-shaft should be as long as possible, to obviate the up-and-down shove on the sliding-bed.

The rods on which the sliding-bed is mounted, running through holes bored in cross-cleats on the under surface of the sliding-bed, are of  $\frac{3}{8}$  inch doweling, and are kept lubricated with graphite. The arrangement for holding the tubes which are to be shaken consists of two tin shields (*c*, Fig. 8) in the form of tubes, each containing a large coiled brass spring, faced with a concave block, *b*. The springs must be soft, and the coils wide, so that they can be compressed into a small space. Opposite each shield on the opposite end of the sliding-bed, is a depression, *a*, to hold the tube steady. Sizes and exact measurements are



Fig. 10.—The laboratory.

not given, as each one will want to construct the apparatus of such a size as best adapted to his own needs.

Everything on this shaking machine with the exception of the motor and the two pulleys, was made by the writer during spare moments, and at very small cost. All of the pieces of apparatus described can be made at odd moments, which are as often as not wasted in some profitless form of so-called recreation. The work is of a character that will provide as much pleasure and recreation for the busy doctor—as much mental rest—as billiards or motion-pictures, provided only that he is interested. Once the apparatus is in his possession, he will find that he has within his reach practically all the laboratory methods performed nowadays as routine clinical procedures, and that the tests which he knows to be so invaluable in his work and which he once considered irksome drudgery, will now take but little of his time and will give him pleasure in their performance.



# RAPID EXAMINATION OF OCCULT BLOOD BY THE BENZIDIN METHOD\*

BY WARREN T. VAUGHAN, M.D.

STOOL examination, a laboratory procedure too often neglected by the general practitioner, and for very obvious reasons, is occasionally the source of a good clue in the diagnosis of morbid processes. It is only in well regulated hospitals, where various facilities make routine stool examination less onerous a task, and where there is always a young interne whose duty it is, and who has no choice in the matter, to examine them, that the procedure is consistently carried out on every patient. Quite obviously, every gastrointestinal case should be attacked from this angle, every ulcer or carcinoma suspect should be repeatedly examined. Occasionally a positive stool test for occult blood in a case, clinically not gastrointestinal, will suggest more thorough examination, and thus permit a correct diagnosis. Such, for instance, are cases of intestinal parasites and carcinoma, in which an early diagnosis is always so important. Barker<sup>1</sup> states that as much as 5% of blood may be present in feces without its presence being recognizable to the naked eye. A very satisfactory hospital routine to be followed, and one that should be strongly recommended to the general practitioner, is the routine examination of at least one stool from every patient, for occult blood, in addition to a simple microscopic examination of an unstained specimen. Of course, more frequent and more complete examinations should be made of stools from cases suspected of having gastrointestinal lesions, whether blood is present or not.

The test most generally employed is the guaiac test, usually Weber's method. The benzidin test as usually described, corresponds in technic, but is considered more delicate; by some even *too* delicate. Perhaps the simplest guaiac test consists in rubbing up fecal material the size of a pea in 5 c.c. of glacial acetic acid; shaking the solution with approximately 10 c.c. of ether; pouring off the supernatant ether, after it has separated out; and overlaying the ethereal extract with freshly prepared tincture of guaiac, to which about one-half its volume of hydrogen peroxide has been added. The blue or violet fading ring at the zone of contact is considered positive. The benzidin test, as generally performed, is done in essentially the same manner, or perhaps a little easier by the method described by Barker,<sup>1</sup> "A small piece of the feces is rubbed up with water; 3 c.c. of this unfiltered suspension is mixed with 2 c.c. of an alcoholic benzidin solution (concentrated by heat and filtered after cooling), and 2 c.c. of a 3% solution of hydrogen peroxide; a few drops of acetic acid are then added. If blood be present, an intense green color appears."

Grunwald<sup>2</sup> reports the relative delicacy of the various tests for occult blood as follows:

Weber's test (his own figures)	-	positive with one part blood in	2000 dilution
Benzidin test	- - - -	positive with one part blood in	150000 dilution
Hemochromogla (spectroscopic test)		positive with one part blood in	5000 dilution
Potassium cyanide	" "	positive with one part blood in	5000 dilution

\*From the Medical Clinic of the Peter Bent Brigham Hospital, Boston, Mass.

Dewis,<sup>3</sup> in 1907, published an excellent review of the literature and a description of the comparative values of the different tests. He says that whereas many substances reacted positively to the original guaiac test, substances such as the oxide and subcarbonate of iron; ferrous salts, and iron sulphide; acetates, citrates and chlorides; the subacetate and sulphate of copper; and pus, all of which give a blue color of varying intensity, yet, as modified by Weber by the use of an acetic acid ethereal extract, most of the interfering substances, particularly the iron preparations, pus, saliva, milk, and starch no longer give positive reaction. Brandenburg<sup>4</sup> has shown that the positive reaction to pus is due to nucleoalbumin, and that it is precipitated by acetic acid. It is this precipitation by the acetic acid-ether extract that prevents the positive reaction to pus. It is found also that the acid-ether combination evidently prevents positive reactions for all common drugs. Weber found that of 67 medicines administered by himself to patients, none reacted positively with his guaiac test.

O. and R. Adler<sup>5</sup>, in 1904, first described the benzidin color test for occult blood, and it is the method as essentially described by them that is positive with one part of blood in a dilution of one to one hundred and fifty thousand parts. This is indeed far too delicate for clinical work, and was so modified by Schlesinger and Holst<sup>6</sup> as to be much less delicate, and yet more sensitive than any of the other blood tests. According to their method, a saturated solution of benzidin is prepared by adding a knife-point of benzidin to 2 c.c. of glacial acetic acid in a clean test tube. This solution is preferably made fresh. A small piece of fecal material is stirred in a small test tube containing about 2 c.c. of water, after which it is brought to the boiling point to destroy the activity of enzymes. The benzidin solution is diluted about three times with commercial 3% hydrogen peroxide. Two or three drops of the boiled feces suspension are transferred to about 3 c.c. of the benzidin-acetic acid-peroxide mixture. The presence of blood is soon indicated by the development of a beautiful green, blue-green, or blue color.

Dewis, in using the test, remarks concerning the question of enzymes giving a positive reaction, that "in 22 specimens of stools, 10 benzidin tests were negative, and the remainder positive. No definite green color change was found *before* boiling, that was absent *after* boiling the specimen; but in nearly all the 10 negative tests, color change was wholly absent without boiling, proving that enzymes seldom, if ever, interfere with the benzidin test in stools." He feels that in testing gastric contents, it would be safer to always boil first as directed in the test.

Wagner<sup>7</sup>, in 1914, recommended the so-called "dry test" for blood—a test which is reliable and extremely simple. He uses as reagent a knife-point of benzidin (powdered)—an amount the size of a match head—2 c.c. of glacial acetic acid, and 20 drops of a 3% solution of hydrogen peroxide. A few drops of this mixture, poured over a little of the solid feces, on a clean glass slide, will produce a greenish-blue fading color if positive. We have found it most convenient to pick up a particle of fecal material the size of a match head on the end of a toothpick and spread it out somewhat on a microscopic slide. Viewed against a white background, the green color stands out strikingly well.

It appears within five or ten seconds, and persists a minute or more. The hydrogen peroxide need be only roughly two-thirds the amount of glacial acetic acid, and the latter only approximately 1 c.c. The only apparatus required is a short test tube for mixing the benzidin, acetic acid and hydrogen peroxide, and a medicine dropper. One or two drops of reagent mixture suffices. It should be freshly prepared, but the solution, or rather suspension, remains good for two or three hours. In lieu of a glass slide any white glazed paper will suffice,—a calling card does very well. If paper is used there is nothing to clean up afterwards.

As regards accuracy and delicacy of reaction, when several months ago we first started to employ this test, it was with the assumption that, the benzidin test being more delicate than the guaiac, a negative benzidin would certainly indicate absence of occult blood, whereas, a positive test should be checked up by a guaiac test. This procedure was carried out for some time; and, even when doing a guaiac test on all stools positive by benzidin tests, much less time was consumed than was formerly done in doing guaiac tests only. Almost invariably, positive benzidins were accompanied by positive guaiacs. In the few cases in which negative guaiacs were obtained, by careful "playing" with the guaiac test (varying the proportions of the components) a definite and strongly positive guaiac was always obtained. We found that varying the amount of guaiac, or of hydrogen peroxide frequently made a negative guaiac become positive to correspond with a positive benzidin. With attention to these points, then, the guaiac and benzidin tests almost always agree, and it was concluded that inasmuch as the guaiac test has more variables which appear to influence its reactivity, any discrepancy between the two tests points to the guaiac test as the incorrect one until it can be proved by repetition to be otherwise. A negative benzidin test is more reliable evidence of the absence of blood from the stools than is a negative guaiac test.

The question is frequently raised as to whether meat ingested, or muscle fibers in the stool will give a positive test for occult blood. Dewis and Cowie<sup>8</sup> both report that an ordinary meat diet rarely gives a positive Weber test. Cowie, however, obtained a definitely positive guaiac reaction after the ingestion of 10 gm. of scraped raw meat, but none from lesser amounts of meat. Lewis conducted a similar experiment in which he ingested a "hearty evening meal of very rare roast beef for two days, other meals being meat free." On the four succeeding days he limited his diet to eggs, salt codfish, potatoes, oatmeal, fish cakes, fresh mackerel, baked beans, bread, butter, tea and coffee. Twenty-four hours after the second meat ingestion, both Schlesinger and Holst's benzidin test and Weber's guaiac test were strongly positive. The benzidin test remained positive after the fecal suspension had been boiled. After forty-eight hours, the benzidin test (both boiled and unboiled) remained positive, while the Weber test had become negative. On the third day the benzidin test was positive (unboiled) and negative (boiled), while the guaiac remained negative. On the fourth day after rare beef ingestion, both benzidin and guaiac tests were negative. This series, besides demonstrating that if sufficient rare meat be ingested a positive test for occult blood will result, also shows very well the relative sensitiveness of the two tests employed.

In contrast to this is Medical case No. 5811, Peter Bent Brigham Hospital, a man admitted with a diagnosis of pernicious anemia, but who on thorough examination showed many additional symptoms, chiefly gastrointestinal, which complicated greatly the diagnosis. Of thirteen stools examined, all gave a negative benzidin test (Wagner), but showed large amounts of fat and many undigested muscle fibers on microscopic examination. The meat eaten, however, was not very rare. A characteristic stool was as follows:

December 28, 1916.

STOOL: Gray, unformed, pasty, shiny. No macroscopic blood, mucus nor parasites. *Microscopic*: Numerous sheaths of fatty acid crystals and, after heating with acetic acid, numerous fat droplets and needle crystals. Numerous muscle fibers with striations well preserved. No starch granules. No pus or ova. Bile + ( $\text{HgCl}_2$  test). *Benzidin*: Negative (Dr. Koefod).

Medical case No. 5377, a case typically pernicious anemia, and with intermittent attacks of diarrhea shows very well the fact that Wagner's benzidin test does not react positively to either muscle fiber or pus:

September 30.

STOOL: Thin, brown, semisolid. No macroscopic blood, pus or parasites. A *microscopic* search revealed no parasitic ova. No starch nor fat globules. No muscle fibers. *Guaiac* test, negative.

October 3.

STOOL: Brown, watery, no macroscopic blood or pus. No segments of worms. *Microscopic* examination reveals no ova or segments—no meat fibers. No blood. Much pus. *Guaiac* test, negative.

October 6.

STOOLS: The last eight stools showed negative *benzidin* test, and on *microscopic* examination, no pus or parasitic ova, but many meat fibers.

October 8.

STOOLS: Of four stools examined, two are unformed and two fluid. Two show some macroscopic mucus. *Color* golden yellow brown. *Microscopic* examination shows very many leucocytes in two of the four stools. *Benzidin* test, negative on all four stools.

October 10.

STOOLS: (Oct. 8, 9, 10) Pus in one of four stools. This is the only stool that has shown a *positive benzidin* test.

October 11.

STOOL: Brown, semisolid, no macroscopic blood, pus, or segments. No *microscopic* blood or ova. Very few meat fibers. Considerable pus. Some phosphates. *Guaiac* negative.

October 17.

STOOL: Semisolid, normal brown. No macroscopic blood, pus or parasites. *Microscopic* examination, few red cells, otherwise negative. *Benzidin*, plus.

November 6.

STOOL: Fluid brown, much thick mucus. One small blood streaked spot. No other gross abnormality. *Microscopically*, blood cells and epithelial cells—nothing else abnormal. Careful search for ova and segments revealed none. *Benzidin*, strongly positive.

November 8.

STOOL: Fluid, dark brown, foul-smelling. Much thin mucus. No macroscopic blood, pus, or parasites. *Benzidin*, negative.

November 17.

Stool: Fluid, chocolate colored, no gross abnormality. *Microscopically*, nothing abnormal seen. *Benzidin* and *guaiac*, positive.

Dewis found that with the Schlesinger-Holst benzidin test the following substances when tested directly reacted positively: raw green vegetables (slight); raw wheaten flour, corn, oatmeal, potatoes, carrot, parsnips, and squash. When cooked, all of these were negative. All meats, cooked and uncooked, gave positive benzidin test. Raw fish, fresh, dry and pickled, and shell fish gave a positive reaction, which became much weaker after cooking. After ingestion of fish, the stool was usually negative, although sometimes positive after fresh fish. Eggs were negative. Blaud's pills were quickly positive, but after ingestion of thirty grains daily there was no positive reaction in the stools.

With Wagner's test, we have found that thoroughly cooked meat still reacts positively, whereas cooked vegetables (potatoes, carrots, tomatoes) are negative. Blaud's pills, tincture of ferric chloride, bismuth subnitrate, potassium iodide, extract of cascara sagrada, compound cathartic pills, and A B & S pills, all reacted negatively, when treated directly with the reagent mixture. The pills were all crushed previous to testing.

As the test is applied to urine examination, urine containing red blood cells, whether it be bloody or merely smoky, reacts positively, whereas, if there be pus alone, no green color is produced.

Since Wagner's dry method was instituted, Doctors Golden, Barrows, Koe-fod, and myself have used it on over eight hundred cases, with entire satisfaction. Probably the benzidin test is more delicate than the guaiac test, and it *may* react when a guaiac test definitely will not; but it is well demonstrated that, for clinical purposes, its reliability and greater ease of execution make it preferable to the guaiac tests, and a negative benzidin test is more reliable evidence of the absence of blood from the stool than is a negative guaiac test.

Gregersen<sup>9</sup>, of Copenhagen, reported in 1916 an exhaustive series of tests for occult blood in the stools. He finds the test positive in practically all of the stools of cancer patients, positive in some stools of ulcer cases; but never constantly positive in the same patient; and constantly negative in uncomplicated chronic gastritis, achylia, colitis, simple dyspepsia and constipation, gall stones, cardiac disease, edema, ascites, nephritis, and hepatic cirrhosis. In all of his work he states that he has relied on the Wagner benzidin test as the most sensitive and reliable method.

Groat,<sup>10</sup> in 1913, published in the *Journal of the American Medical Association* a benzidin test, nearly as simple as the Wagner method, in which the feces were suspended in water, and barium dioxide was used instead of hydrogen peroxide.

A slight variation of Wagner's test (Dudley Roberts' test) is now being placed on the market in a very usable form by Squibb and Sons. This is described in the *Journal of the American Medical Association*, September 16, 1916, as follows: "This consists of tablets each containing 5 grains of a trituration of benzidin, 1 part, and sodium perborate 20 parts, and glacial acetic acid (supplied in boxes containing 100 tablets in vials, and a bottle of glacial

acetic acid). A tablet is placed in a small saucer or other suitable container; to it is added a quantity of the material to be tested (a weak solution of the stool or stomach contents or urine), sufficient to wet thoroughly, but not to cover the tablet entirely, and then a drop or two of the acetic acid is allowed to fall on the tablet; if blood is present, the tablet will turn greenish blue, the extent of the coloration and the time of its development depending upon the amount of blood present. (To avoid contamination of the glacial acetic acid remaining in the bottle, care should be taken not to touch the tablet or specimen with the rod which is used to transfer the acetic acid for the test.)"

The cooperation of the attendants of a patient in the procuring of a stool specimen is perhaps more spontaneous if the physician leaves with them the means of easily obtaining it. Very good for this purpose is one of the ordinary wooden tongue depressors,—to be used as a spatula—and a small porcelain ointment or salve jar with metal screw cap,—as a container.

#### CONCLUSIONS.

The Wagner benzidin test for occult blood is recommended on account of its ease, rapidity, and comparative cleanliness in execution, and on account of its reliability.

As carried out, this benzidin test is not too delicate for clinical use.

A negative benzidin test is more reliable evidence of absence of blood than is a negative guaiac test.

Meat fibers, pus, and the usual drugs and foods ingested do not interfere with the reaction. The ordinary hospital diet, with meat, gives a negative reaction; but, if sufficient quantities of rare meat be taken, the stool reaction may become definitely positive.

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## EDITORIALS

### *The Relation of Body Surface to the Basal Metabolism of the Body*

THE observations that small animals require a greater amount of food than do larger ones of the same species, and that children demand a greater food supply than adults, has led to much speculation as to what determines the energy requirements of the body. Years ago Rubner furnished experimental data which apparently proved that the relative metabolism of all mammals when reduced to terms of surface area was almost alike. If the body surface bears a casual relationship to the energy requirements of the body, the rate of cooling must be the determining factor. It can hardly be that such a relationship, if it does exist, can be casual, for the amount of heat lost through the lungs is considerable, and Rubner's law fails to take consideration of this.

Nevertheless, for years the standard textbooks of physiology have taught that such a relationship exists, and that the metabolism of the resting individual is proportional to the surface area and not to the body weight. Recent investigations of the metabolism of the body under conditions which give the lowest possible rate of energy consumption (nasal metabolism) have awakened a new in-

terest in the application of Rubner's law. If such a law correctly expresses the relative metabolism, a fixed figure representing the caloric output per hour and per square meter of body surface for normal people should furnish a valuable index for comparison with figures obtained similarly in cases of patients suffering from pathological conditions.

The observations of a number of investigators indicate that the average basal metabolism of adults in normal health amounts to about forty calories per hour and per square meter of body surface. This figure represents the average basal metabolism found in a large number of determinations in which the surface area of the body was most carefully estimated, and serves as a normal control for practically all the recent work on metabolism in disease.\*

While it is no doubt true that the metabolism of the body varies more nearly directly with the body surface than with the body weight in normal adults, in infants Benedict and Talbot<sup>1</sup> failed to confirm the law. Murlin and Hoobler<sup>2</sup> although not agreeing with the conclusion of the above authors, conclude that there is no good reason for estimating the food requirements of the infant on the basis of surface area rather than on the basis of weight.

Recently Benedict<sup>3</sup> has added some very interesting observations on the changes occurring in the metabolism of a man during a fast of thirty-one days. By adapting the principles<sup>4</sup> of body surface measurement so admirably worked out by DuBois and Du Bois,<sup>5</sup> he was able to compute the surface area of a man whom he previously had thoroughly studied during a long fast, from a series of photographs taken at regular periods during the period of starvation. The surface area thus obtained allowed him to recalculate the metabolism of the man in terms of surface area and calories per hour. He found that during the fast the metabolism decreased twenty-eight per cent. He believes that this furnishes proof of the inapplicability of the surface area law when applied to subjects with widely varying states of nutrition, and concludes that a normal figure obtained from a large number of healthy subjects does not furnish a correct standard for comparison with that obtained in cases where there is a marked emaciation as in diabetes.

In this individual he failed to find any relationship between heat production and surface area, and again affirms his belief that the active mass of protoplasmic tissue rather than the surface area is one of the greatest factors in determining the metabolic activity of an animal.

It may be as Benedict and Talbot suggest, that the active mass of protoplasmic tissue develops proportionally to the body surface, and that therefore in normal individuals the body surface, while in no way determining the metabolism of the body, affords a measure of that factor which does play the major role in determining the body requirements.

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\*See editorial, *Jour. Lab. and Clin. Med.*, Sept., 1916, i, 928.



# *The Journal of Laboratory and Clinical Medicine*

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## ORIGINAL ARTICLES

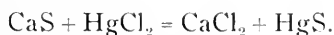
### CALCIUM SULPHID, AS THE CHEMICAL AND CLINICAL ANTIDOTE FOR MERCURIC CHLORID POISONING, WITH EXPERIMENTS AND CASE REPORTS\*

By J. H. WILMS, M.D., CINCINNATI, OHIO.

**A**N effective antidote should render a poison innocuous. A chemical compound has an antidotal action when its elements are loosely enough combined, that upon coming in contact with another chemical compound in the human body, their elements have so great an affinity that new compounds are formed which are inert. Once this union takes place the tissues are relieved of the destructive elements of the poison, and regenerate and resume their physiological function. It has often been claimed that an organ, once damaged, cannot regenerate. This, however, has been disproved by the following experiments:

My first experiment demonstrated to me that a clinical as well as chemical antidote has been found in calcium sulphid. This has been verified in repeated experiments by myself and others.

A hypothetical explanation may be found in the following simple, approximately accurate equation:



Mercuric sulphid (HgS) and calcium chlorid ( $\text{CaCl}_2$ ) thus formed are two almost inert chemical compounds. This is the test tube chemical reaction and forms the hypothesis of my animal experimentation.

#### THE METHOD OF POISONING THE ANIMAL WITH BICHLORID OF MERCURY.

A compressed tablet containing mercuric chlorid, 7.3 gr. and ammonium chlorid 7.7 gr., which I thought was the most insoluble tablet on the market, was used. It was so hard that in a half hour it would not begin to dissolve

\*From The B. Merrill Ricketts Experimental and Surgical Research Laboratory, Cincinnati, Ohio

in a half pint of water without agitation. Some tablets dissolve in a half minute in the same amount of water, and dissolve in less time in a one per cent hydrochloric acid solution.

The tablet was thrown into the dog's throat, the mouth of the animal being held wide open. At times the tablet would be swallowed whole, and sometimes chewed and then swallowed. The variations in solubility in the stomachs of the different dogs were as follows:

ANIMAL.	AMOUNT OF TABLET VOMITED.	TIME INTERVENING BETWEEN TAKING AND VOMITING PART OF TABLET.	
Dog No. 1	1/3 tablet, (2 gr. $\text{HgCl}_2$ )	5	minutes
Dog No. 2	.1 tablet, (.75 gr. $\text{HgCl}_2$ )	16	"
Dog No. 3	None (but profuse vomiting)	4	"
Dog No. 4	.1 tablet (.75 gr. $\text{HgCl}_2$ )	3.5	"
Dog No. 5	1/3 tablet (2 gr. $\text{HgCl}_2$ )	3	"
Dog No. 6	None (but profuse vomiting)	2.5	"
Dog No. 7	1/3 tablet (2 gr. $\text{HgCl}_2$ )	4	"
Average solubility 6 gr. $\text{HgCl}_2$ in 5 to 6 minutes.			

This table will show the unusual solubility in the dogs' stomach of the very insoluble tablets.

In the experiments which follow, the dogs received no antidote. The experiments show the extensive destructive action of bichlorid of mercury in dogs.

#### EXPERIMENT NO. 1.

*Nov. 2, 1915.*—White bull terrier, weight 16 lbs. (No antidote).

One tablet of bichlorid of mercury (7.3 grains) and ammonium chlorid (7.7 gr.) was thrown down the dog's throat. Vomiting commenced in a few minutes; severe intestinal tenesmus and strangury followed.

*Nov. 3, 1915.*—Dog was listless.

*Nov. 4, 1915.*—Dog listless and lay in a cramped position.

*Nov. 6, 1915.*—Dog very sick.

*Nov. 7, 1915.*—Dog found dead.

*Postmortem.*—Intestines were red and inflamed; stomach contained a thick coffee-colored fluid, a round ulcer about one centimeter in diameter was found on the anterior surface near the pylorus; submucous clot-like masses were found about the pylorus, varying in size from two to five millimeters in diameter. The duodenum was red and inflamed; bile, thick and gritty; the kidney cortex was red and streaked; the medulla nephrica was congested, proportion cortex one to medulla three; red fluid in the calices. *Chest.*—Both lungs hepatized. *Heart.*—Ulcer on the visceral layer of the pericardium over the terminals of the coronary vessels; pericardium thickened. Myocardium inflamed; muscoli pectinati markedly inflamed; subendocardial ecchymoses in both ventricles; esophagus inflamed.

#### EXPERIMENT NO. 2.

*May 12, 1916.*—Small white dog, 15 lbs. (No antidote).

4:35 P. M. A 7.3 gr. bichlorid of mercury tablet thrown down dog's throat.

4:37 P. M. Slight blood-stained vomiting was followed by a frequent vomiting of thick, yellow, glary mucus.

*May 15, 1916.*—Dog found dead in the afternoon. Postmortem findings similar to those of Experiment No. 1. (Specimens were used for chemical analysis.)

#### EXPERIMENT NO. 3.

*May 16, 1916.*—Yellow setter, 40 lbs. (No antidote).

4:56 P. M. A 7.3 gr. tablet bichlorid of mercury thrown down throat.

4:56.75 P. M. Dog restless.

4:57.5 P. M. Dog licks chops.

4:58.5 P. M. Nausea.

4:59 P. M. Vomiting and violent straining; vomitus cruentus; stringy and tenacious mucus vomited in large quantities.

5 P. M. Vomited  $\frac{1}{3}$  of tablet.

5:01 P. M. Readministered the piece of tablet.

5:02 P. M. Vertigo; very sick.

May 20, 1916.—Dog killed for specimens.

Postmortem revealed similar condition to first experiment with the exception that the stomach was very much corroded. (Specimens used for chemical analysis.)

#### EXPERIMENT NO. 4.

This experiment will show that enough mercury is vomited and excreted to poison another dog.

A medium-sized dog was placed in the cage which had been occupied by the dog in Experiment No. 7. The dog died six days after being placed in the cage, after showing symptoms of mercury poisoning: i.e., vomiting and diarrhea.

*Postmortem*.—Pregnant bitch; lungs, hypostatic pneumonia; duodenum, inflamed and extensive ecchymoses of the submucosa. Stomach inflamed and ecchymosed submucosa. Bile dark. Kidneys swollen, dark red, capsule peels off, edges pout when cut. Cecum and ascending colon contain many pin-head ulcers. Heart contained a fatty-looking thrombus that was attached to the walls of both ventricles.

After this experiment we saw that it was necessary to antidote the cages and all the other animals, in the laboratory, with calcium sulphid. This accounted for the death of several dogs that had died of chronic mercurialism.

The following experiments will show the effect of the antidote, calcium sulphid, on the dogs that received bichlorid of mercury.

#### EXPERIMENT NO. 5.

Oct. 16, 1915.—A good sized dog, about 30 lbs. in weight, with a brown woolly coat. 4 P. M. one tablet containing 7.3 gr. of bichlorid of mercury and 7.7 gr. chlorid of ammonia was given per mouth. In a few minutes the dog vomited. This was followed by frequent vomiting and rectal tenesmus.

Oct. 18, 1915.—Dog thin and sick; bloody diarrhea. Forty-eight hours after receiving bichlorid of mercury, with no antidote in the interim, a solution of calcium sulphid which had been boiled and filtered through cotton (7.5 grains CaS to 7.5 ounces of tap water) was administered intravenously.

Oct. 20, 1915.—Dog much improved.

Oct. 30, 1915.—Dog well and lively.

Nov. 4, 1915.—Dog, playful, looks clean and has a healthy glossy coat.

Nov. 8, 1915.—Dog very lively.

Nov. 17, 1915.—Unusually lively.

Nov. 29, 1915.—Reported the experiment to the Cincinnati Academy of Medicine and presented the dog.

Dec. 2, 1915.—Killed the dog by bleeding.

*Postmortem*.—Dog well nourished and fat. Tissues healthy in appearance. Kidneys clear and normal microscopically. Liver paler than normal (due, perhaps, to the hemorrhage). Bile thin and amber-colored. Stomach and intestines normal.

#### EXPERIMENT NO. 6.

March 31, 1916.—White dog, mongrel, weight 19 lbs.

4:13.5 P. M. 7.3 grs. bichlorid of mercury tablet per mouth.

4:15 P. M. Restless.

4:16 P. M. Straining, urinated profusely; dark colored urine.

4:17 P. M. Vomiting thick tenacious mucus.

4:18.5 P. M. Violent retching; vomitus bile-stained.

4:27 P. M. Vomitus cruentus.

4:28 P. M. Bowels moved.

4:29 P. M. Straining to stool; rectal tenesmus; a small piece of tablet vomited, which was readministered.

*April 1, 1916.*—Dog very sick and listless.

*April 2, 1916.*—10 A. M. So very sick, dog was expected to die.

4 P. M. Antidote, a solution of calcium sulphid, 7.5 gr. to 7.5 ounces of water, the solution boiled and filtered through cotton, was administered intravenously. About an ounce of the solution was lost during the operation. While injecting the antidote the dog made several gasps.

*April 4, 1916.*—Dog much improved.

*April 6, 1916.*—Dog apparently normal.

*April 28, 1916.*—Dog normal and lively.

*June 6, 1916.*—Dog killed.

*Postmortem.*—All organs macroscopically normal. Mr. R. A. White, curator of the laboratory, and several pathologists examined, microscopically, specimens of the kidneys and reported normal kidneys.

#### EXPERIMENT NO. 7.

*April 8, 1916.*—Black water Spaniel.

3:53.5 P. M. One tablet bichlorid of mercury (7.3 grains) administered by mouth.

3:57 P. M. Vomited.

3:58 P. M. Blood-stained vomitus.

4:08 P. M. Rectal tenesmus and stool.

*April 9, 1916.*—Dog very sick.

*April 10, 1916.*—Dog lies quietly.

*April 11, 1916.*—4 P. M. 72 hours after receiving the bichlorid of mercury, the antidote was administered intravenously (a solution of calcium sulphid, 7.5 grains in 7.5 ounces of water, boiled and filtered through cotton).

*April 14, 1916.*—Dog died some time during the night.

*Postmortem.*—Lungs, double pneumonia. Pericardium thickened. Heart contained large fatty-looking thrombus, attached to the right ventricular wall. The left ventricle showed subendocardial and myocardial ecchymoses. The stomach mucous membrane was ulcerated, involving the whole lesser curvature. Esophagus pale. Small intestine had many ulcers; large intestines had ecchymoses and bloody stool. Kidney, cortex yellow, left kidney markedly congested; liver congested, (venous). Bile, dark and thick.

#### EXPERIMENT NO. 8.

*April 20, 1916.*—Hound.

3:44 P. M. 7.3 grains of bichlorid of mercury tablet administered by mouth.

3:47.5 P. M.—Vomiting; a small piece of tablet vomited which was readministered per os. This was followed by frequent vomiting of tenacious and glary mucus.

3:53 P. M.—Rectal tenesmus.

3:54 P. M. Strangury and rectal tenesmus.

*April 22, 1916.*—4 P. M. Solution of 7.5 gr. calcium sulphid in 7.5 oz. water was administered intravenously, into the external jugular vein.

*April 23, 1916.*—Dog not so well as others.

*April 24, 1916.*—No improvement.

*April 25, 1916.*—Dog looks very sick.

*April 26, 1916.*—Bloody stool.

*April 28, 1916.*—Stool bloody.

*April 30, 1916.*—Dog died during night.

*Postmortem.*—Dog emaciated, bloody stool. Stomach dilated and inflamed; contained a brownish-green fluid; inflammation and ecchymoses of the rugae. Duodenum inflamed. Gall-bladder thickened and bile thick. Lungs somewhat congested. Heart contained a fatty-looking thrombus attached to the walls of the right ventricle.

Later it was discovered that the calcium sulphid used in this and experiment No. 7 was an old stock solution which had been in the laboratory for some time, and had lost most of its sulphur. Since sulphur is the active agent in the antidote, the solution was of very little value. This was borne out by a number of experiments, in which the same solution was used.

Wilhelm Ostwald gives the following explanation showing the chemical change in the old solution. "The salt . . . is only very slightly soluble

in water, but is decomposed on being warmed for some time with it, calcium hydrosulphide passing into solution and calcium hydroxide remaining behind:  
 $2 \text{CaS} + 2 \text{H}_2\text{O} = \text{Ca}(\text{HS})_2 + \text{Ca}(\text{OH})_2$ ."

## EXPERIMENT NO. 9.

*May 12, 1916.*—Large dog, setter.

4:25 P. M. A 7.3 grains tablet bichlorid of mercury given per mouth.

4:28 P. M. Vomiting. 1/3 of tablet found in vomitus. This piece was readministered. This was followed by frequent vomiting.

4:42 P. M. Stool. Rectal tenesmus.

4:47 P. M. Blood streaked vomitus.

*May 14, 1916.*—4:25 P. M. Antidote, 7.5 grains calcium sulphid in 7.5 oz. water, after being boiled and filtered, injected intravenously.

*May 16, 1916.*—Dog lively.

*May 18, 1916.*—Dog apparently well.

*May 24, 1916.*—Steady gain in weight.

*June 6, 1916.*—Dog killed.

*Postmortem.*—Dog well nourished and fat. Organs appeared to be normal. Kidneys examined microscopically by Mr. R. A. White and pronounced normal.

This experiment was done to test the stability of the oily suspension of calcium sulphid, and it proved to be as unstable as the watery solution. The same preparation was used 2 weeks later, and the dog it was used on died of clonic convulsions in about two hours and a half after it received the injection.

## EXPERIMENT NO. 10.

*July 17, 1916.*—9:05 A. M. 7.5 grains of  $\text{HgCl}_2$  administered.

3:45 P. M. Calcium sulphid given intravenously, 7.5 grains to one ounce of sweet almond oil.

The dog steadily improved.

*Aug. 10, 1916.*—The dog is fat and healthy.

## SUMMARY OF THESE EXPERIMENTS

Dog of Ex. 5, 48 hour antidote, recovered.

Dog of Ex. 6, 48 hour antidote, recovered.

Dog of Ex. 7, 72 hour antidote (old calcium sulphid solution), death on 6th day.

Dog of Ex. 8, 48 hour antidote (old calcium sulphid solution), death on 10th day.

Dog of Ex. 9, 48 hour antidote, recovered.

Dog of Ex. 10, 6 hour antidote ( $\text{CaS}$  in oil). Recovered.

These experiments showed to me so clearly the value of calcium sulphid as an antidote for mercuric poisoning that demonstrations were discontinued.

The object of the following experiment is to show the effect of mercuric sulphid, when administered by mouth.

## EXPERIMENT NO. 11.

*May 8, 1916.*—3:30 P. M. A solution of 7.5 grains of bichlorid of mercury was mixed with 7.5 grains of calcium sulphid in a test tube and the resulting solution was thrown down a dog's throat. No symptoms developed, with the exception of a slight vertigo, which may not have been the result of this solution.

*May 24, 1916.*—Dog very well and lively, having shown no symptoms at any time.

*May 31, 1916.*—Dog used for other experimental purposes. No symptoms were present.

The following experiment was performed to determine the immediate effect of introducing a calcium sulphid solution intravenously.

## EXPERIMENT NO. 12.

Oct. 12, 1915.—A rabbit received intravenously, one grain of calcium sulphid to one ounce of water, and showed no immediate symptoms.

Oct. 16, 1915.—Rabbit normal

Oct. 30, 1915.—Rabbit normal.

Nov. 9, 1915.—Rabbit died, after receiving a dip.

*Postmortem*.—Animal thin and emaciated. Abdominal and thoracic cavity negative. It is hardly possible that the calcium sulphid was the cause of the rabbit's death.

The following is a résumé of experiments performed by Mr. Frank Maltaner, M.A. of the laboratory of the Cincinnati Board of Health, to verify my experimental work with calcium sulphid. These experiments were done at the instigation of Dr. J. H. Landis, Health Officer of the city of Cincinnati. Quoting Mr. Maltaner:—

“NOTES ON THE EFFECT OF BICHLORID OF MERCURY ON RABBITS, AND THE VALUE OF THE CALCIUM SULPHID ANTIDOTE USED ON THESE ANIMALS.

*Administration of the Poison*.—The chemically pure bichlorid of mercury was filled into gelatin capsules and forced down the animal's throat. After taking a lethal dose of the poison, the animals refused to eat or drink. Profuse defecation usually occurs, followed after about two hours by more or less diarrhea. There is continued refusal of food and diarrhea and gradual loss of weight, followed by death in from several hours to four days.

Examination of the cadaver shows usually some fluid in the peritoneal cavity (not constant). The lungs sometimes show acute inflammatory areas. The veins of the peritoneum, stomach, mesentery, and heart are injected. The small intestine has a greyish, metallic color and putty-like consistency. The kidneys show more or less swelling, and are greyish white in color and embedded in an extensive fatty capsule. The spleen is small and metallic. The stomach is large and tense, and the inner coating is much inflamed and corroded.

Mercury was detected in the heart, liver, spleen, kidney, blood, and urine. Mercury was also detected in the blood drawn from the marginal vein of the ear, 15 minutes after the administration of the bichlorid by mouth.

The lethal dose of the bichlorid, when administered by mouth, proved to be .25 grain per pound of animal. This dose invariably killed in from 12 hours to 4 days. A dose of .2 grain per pound or less had no apparent effect upon the rabbits.

*Administration of the Antidote*.—The antidote is an aqueous solution of calcium sulphid in a dilution of one grain to the ounce of distilled water. It is prepared by dissolving the calcium sulphid in boiling water and filtering hot. It is then ready for use. We used both the product prepared for Dr. Wilms by the Merrill Chemical Co. and some prepared ourselves with equal success.

The dosage adhered to was the same as recommended by Dr. Wilms: viz., one grain of the antidote to each grain of bichlorid taken. It was given intravenously, the marginal vein of the ear being selected in each case.

Two rabbits that received .25 grain per pound weight of bichlorid were given the antidote after 2.25 hours and 5.75 hours respectively and recovered completely.

"One rabbit received .5 grain per pound weight of bichlorid. The antidote was given after 2.75 hours with complete recovery of the animal.

"One rabbit received .25 grain per pound weight of bichlorid and was given the antidote 5.5 hours later. This rabbit died after 3 days showing typical bichlorid poisoning symptoms and pathology.

"CONCLUSIONS.—The calcium sulphid antidote is certainly of value in the treatment of rabbits poisoned with bichlorid of mercury.

"The antidote must be given, if possible, in less than five hours after the poison has been administered in rabbits.

"The poison is very rapidly absorbed, appearing in the blood (taken from the ear) in less than 15 minutes.

"REMARKS.—The value of the antidote probably lies in the formation of the relatively insoluble, double salt mercuric sulphid mercuric chlorid ( $\text{HgS} \cdot \text{HgCl}_2$ ) and the very insoluble mercuric sulphid.

"Both of these substances are formed in the test tubes, the formation of the insoluble sulphid depending upon the excess of calcium sulphid used."

The following are Mr. Maltaner's Experiments:

#### RABBIT NO. 1.

Rabbit of 2 pounds weight; received .5 grain of mercury bichlorid at 10:40 A. M. April 24.

2 P. M. Diarrhea.

4 P. M. Received .5 gr. of calcium sulphid intravenously.

April 26, A. M. 1-2/3 pounds weight.

April 27, A. M. Dead.

*Postmortem*.—Serous fluid in peritoneal cavity; veins of stomach, heart, mesentery, and peritoneum injected; small intestine has greyish metallic appearance and putty-like consistency; lungs inflamed; kidneys greyish white swellings; spleen small and metallic; stomach tense and inner coating much inflamed and corroded.

#### RABBIT NO. 2.

Rabbit of 2 2/3 lbs. weight received .67 grain of mercury bichlorid at 11 A. M. on April 24.

2 P. M. Diarrhea.

April 25, A. M. Found dead

Postmortem appearance similar in every way to first rabbit.

#### RABBIT NO. 3.

Rabbit of 2 lbs. weight received .5 grain of mercury bichlorid at 9:26 A. M. April 27. 11:45 A. M. Received .5 grain solution of calcium sulphid intravenously.

1:45 P. M. Had slight diarrhea.

April 28, A. M. In good condition and weighed 1 2/3 lbs.

May 5. Complete recovery; weight 2 lbs.

May 18. Weight 2 1/3 lbs.

#### RABBIT NO. 4.

Rabbit of 3 lbs. weight received .75 grain of bichlorid of mercury at 9:40 A. M. April 27.

3:25 P. M. Received .75 grain of calcium sulphid solution intravenously.

April 28, 8 A. M. Slight diarrhea; slight loss in weight.

April 29. Regained weight; good condition.

May 18. Good condition; weight 3 lbs.

#### RABBIT NO. 5.

Rabbit of 4 lbs. weight received 2 grains of bichlorid of mercury at 9 A. M., May 3. 11:45 A. M. Received 2 grains of calcium sulphid solution intravenously.

2 P. M. Diarrhea, profuse and bloody.

May 4, 8 A. M. No diarrhea; weight 3 2/3 lbs.

May 12. Good condition; normal weight.

June 2. Good condition; normal weight.

#### RABBIT NO. 6.

Rabbit of 4 lbs. weight given 1 grain of bichlorid of mercury at 9:35 A. M., May 8.

10:30 A. M. Diarrhea.

May 9, A. M. Dead.

Postmortem condition similar to rabbit No. 1; heart, liver, spleen and kidneys gave chemical test for mercury.

#### RABBIT NO. 7.

Rabbit of 3 lbs. weight received 1.5 grains of bichlorid of mercury 10 A. M., May 9.

May 10, A. M. Dead.

Postmortem same as rabbit No. 1; mercury was detected in the blood taken from ear vein 15 minutes, 30 minutes, 45 minutes after the bichlorid was given by mouth; the heart, liver, spleen, kidney, blood and urine gave chemical tests for mercury.

#### RABBIT NO. 8.

Rabbit of 3 lbs. weight given .15 grain of bichlorid of mercury at 3:05 P. M., May 15.

May 26. No reaction; weighs 3.5 lbs.

#### RABBIT NO. 9.

Rabbit of 3 lbs. weight given .3 grain of bichlorid of mercury at 3:05 P. M., May 15.

May 26. No reaction; weighs 3.5 lbs.

#### RABBIT NO. 10.

Rabbit of 3 lbs. weight given .45 grains of bichlorid of mercury on May 18.

June 2. No reaction; gain in weight.

#### RABBIT NO. 11.

Rabbit of 3 lbs. weight given .6 grain of bichlorid of mercury on May 18.

June 2. No reaction; gain in weight.

#### RABBIT NO. 12.

Rabbit of 3 lbs. weight given .75 grain of bichlorid of mercury on May 26.

May 27. Lost .5 lb. in weight.

May 30. Dead.

Typical mercury poisoning.

#### RABBIT NO. 13.

Rabbit of 3 lbs. weight received .9 grain of bichlorid of mercury on May 26.

May 27. Lost .5 lb. in weight.

May 29. Dead.

Typical mercury poisoning.

#### CASE REPORTS.

In June, 1915, through the courtesy of Dr. Oscar Berghausen, I was called to the Cincinnati General Hospital to treat a case of bichlorid of mercury poisoning. Upon my arrival at the institution at 1 P. M. I found a female patient had taken 110 grains of bichlorid of mercury the evening before with suicidal intent. She was about 30 years of age, and in a moribund condition. After the exhibition of five grains of calcium sulphate by mouth every half hour for three or four hours, she died. This case suggested to me that if I could have given her a solution of calcium sulphid intravenously I might have been able to neutralize the bichlorid of mercury, and that the neutralization would have been rapid enough to have saved her.

On April 19, 1916, I was consulted by Mr. S. of Los Angeles, Cal., whose wife had used by mistake 30 hours previously a 7.5 gr. of bichlorid of mercury tablet intravaginally, thinking it was an antiseptic wafer. The antidote formula, 1 grain of calcium sulphid to 1 ounce of water was telegraphed to her physician, Dr. C. W. Decker.

The patient was given intravenously 7.5 grains of calcium sulphid dis-



solved in 7.5 ounces of water, the solution having been boiled and filtered through cotton. After several days she began to improve and made an uneventful recovery.

On May 25, 1916, Dr. L. N. Denman informed me that he had a patient who had used a 7.5 grain bichlorid of mercury tablet intravaginally to prevent pregnancy. He saw her a short time after the tablet had been introduced, and made a careful examination of the vagina for the tablet, but was unable to find it. Her vagina and vulva were swollen and inflamed. He prescribed one grain of calcium sulphid per mouth every two hours and recommended a douche, 1 dram of calcium sulphid to the ounce of water per vagina. He saw this woman twice after taking the bichlorid tablet and then not again till July 16, 1916. She had been perfectly well in the meantime and had gone away on a trip.

The report of these two cases should be compared with three cases reported by Dr. G. B. Schildecker, American Journal of Obstetrics, 1911, lxi, 473-475.

*Case I.*—A physician made a careful examination 35 minutes after a bichlorid of mercury tablet had been introduced by a woman into her vagina to prevent pregnancy, and he could find no trace of the tablet. Patient died in four days. The postmortem showed parenchymatous degeneration of the liver; marked fatty degeneration of both kidneys; marked fatty degeneration was noticed along the nutrient vessels of the heart. Treatment was not mentioned.

*Case II.*—One tablet of bichlorid of mercury was inserted into the vagina to prevent pregnancy. Treatment was enteroclysis. The patient lived two weeks, died of perforation of the colon with peritonitis. Autopsy showed similar condition to Case I.

*Case III.*—One tablet of bichlorid of mercury was introduced into the vagina to prevent pregnancy. Treatment, vagina was washed out with hot water in twenty minutes, followed by enteroclysis of saline solution. Patient died on the seventh day. Autopsy showed same results as the other cases and passive hemorrhage in the most dependent portions of the peritoneal cavity. A large amount of bloody serous exudate beneath the brain and membranes.

His conclusions were that the tablet was absorbed in twenty minutes, and that the tablet should be removed in a few minutes. The person suffering from such an accident has a small chance of recovery because of the far-reaching effects of the poison, as shown by the autopsy findings in these cases.

On April 1, 1916, through the courtesy of Dr. J. H. Landis of the staff of St. Mary's Hospital, Cincinnati, Ohio, I was permitted to use calcium sulphid intravenously on a man, 39 yrs. of age, who had taken 7.5 grains of bichlorid of mercury the preceding night about 1 A. M. The following day when I saw the man at 3:30 P. M., he had a bloody diarrhea and suppression of urine, accompanied by a great deal of pain in the abdomen, and a very sore mouth. He informed me that he had vomited five minutes after taking the tablet. I gave him intravenously, a freshly prepared solution of calcium sulphid which had been boiled and filtered through cotton, 7.5 grains to 7.5 ounces of water. The next day he was so much improved that his bowel symptoms had prac-

tically disappeared. He had had no stool after the injection, and was passing a normal amount of urine. The second day after the injection he was suffering from a pyorrhea alveolaris and stomatitis, for which I prescribed a grain of calcium sulphid per mouth, every hour. He made an excellent recovery and I presented him to the Cincinnati Academy of Medicine ten days after he had taken the bichlorid of mercury. He informed me that he had gained seven and a half pounds in weight in ten days.

On April 27, 1916, Dr. Hays was called to treat a girl, A. D., 20 yrs. old, who had taken a 7.3 grain bichlorid of mercury tablet by mistake for a headache tablet at 6:30 A. M. Shortly after, she became very sick and vomited. He sent her to St. Mary's Hospital where she received a gastric lavage at 8:30 A. M., after which she was given calcium sulphid, one grain every hour. The second day she went home from the hospital and showed no further symptoms.

On June 2, 1916, a 19 year old girl was supposed to have taken two bichlorid of mercury tablets of 7.3 grains each, with suicidal intent. She became very sick shortly afterward and was found wandering in a sort of delirium. She was taken to St. Mary's Hospital and given one grain of calcium sulphid per mouth every two hours, prescribed by Dr. Hays. The next day she was very sick, but the second day after receiving the calcium sulphid, she was so much improved that she was able to leave the hospital. Her recovery was complete and uneventful.

I was consulted by Dr. F. A. Ireton of Newtonsville, Ohio, on May 21, 1916, concerning Mrs. W., aged 35, who had taken one 7.3 grain bichlorid of mercury tablet. She vomited in about ten minutes. He commenced using calcium sulphid about twelve hours after she had taken the tablet. Tenderness was marked all over the abdomen and she passed pieces of mucous membrane from the rectum. Urinary symptoms were not noted. He reports to me on August 2, that she was dismissed June 1, having made an excellent recovery and that he saw her in his office July 5, when he saw no symptoms of the poisoning.

In January, 1916, Dr. J. Kelley Brammer of Cincinnati, Ohio, administered calcium sulphid by mouth, 7.5 grains, to a woman who had taken a 7.5 grain bichlorid of mercury tablet. August 3, 1916, he reports to me in a personal communication that she made an excellent recovery and showed no untoward effects of the poisoning.

I have information of a number of other recoveries following the use of calcium sulphid in bichlorid of mercury poisoning, but the reports of the consulting physicians were too brief or vague to be herein reported.

I should like to call attention to the possible value of calcium sulphid as an antidote for industrial chronic mercurialism found among felt hat makers, workers in mercury, furriers, dentists, etc. In these cases an occasional dose of calcium sulphid should completely neutralize all the mercury.

## SUMMARY AND CONCLUSIONS.

In advanced cases of mercuric chlorid poisoning the intravenous method of injecting calcium sulphid solution grain for grain of the bichlorid of mercury taken, is the safest and most rapid. An advantage of the intravenous method lies in the assurance that the patient receives the required amount of the antidote, and that it is more direct. Calcium sulphid may also be administered by mouth when the intravenous method is not practicable. When the discomfort or the condition of the patient warrants it, both methods may be used. The use of calcium sulphid by mouth may be continued till all symptoms of mercurialism have disappeared, since it is nontoxic. Recovery has taken place, when the antidote was administered by mouth, as late as twelve hours after taking 7.5 grains of bichlorid of mercury, and thirty hours when administered intravenously.

The minimum lethal dose of bichlorid of mercury in the human being is one decigram. (Barbour.)

The calcium sulphid solution must be freshly prepared for intravenous exhibition or it loses its sulphur and becomes ineffective. Calcium sulphid solution should not be used stronger than one grain to the ounce of water. The solution should be boiled and filtered through cotton. It should preferably be given in the median basilic or the median cephalic vein.

Calcium sulphid is the quickest, simplest and surest antidote for mercurial poisoning used at the present time. It has been proved clinically and in the laboratory.

Experiments showed that mercuric chlorid poisoning produced a wide pathogenesis notably, gastritis, ulcer of the stomach, hepatitis, duodinitis, colitis, endocarditis, ulcerative pericarditis, pneumonitis, tubular nephritis (which is manifested by complete suppression of the urine) and a number of other very marked pathological changes, microscopically and macroscopically manifested. All these tissue changes become normal with the exhibition of one dose of calcium sulphid in solution intravenously grain for grain of poison taken.

When given by mouth, it should be administered in the tablet or crude drug form, in two to five grain doses every hour, until an excessive amount is taken. A grain for a grain is four times the amount of calcium sulphid necessary to neutralize mercuric chlorid in the test tube.

The calcium sulphid solution, if deteriorated, will produce severe convulsions owing to the free action of calcium on the spinal cord hence the necessity of a fresh solution. Another danger is the possibility in deteriorated solutions, of hydrogen sulphid which is a fatal poison being present.

Local antidoting in the stomach by the use of whites of eggs and lavage with large quantities of water is useless; bichlorid of mercury is so rapidly absorbed from the stomach that very little remains in the stomach unabsorbed at the end of five minutes. The vomiting, by this time, is so profuse that if any free bichlorid of mercury were still present in the stomach, it would not remain long enough to cause further damage.

## CALCIUM SULPHID.\*

The product commonly known as calcium sulphid or sulphurated lime is a mixture containing calcium sulphid, calcium sulphate and carbon, and is usually prepared by heating calcium sulphate with charcoal and starch to a red heat. The reaction is



The  $\text{CO}_2$  and  $\text{CO}$  pass off as gases, leaving calcium sulphid and some unchanged calcium sulphate and carbon.

When calcium sulphid ( $\text{CaS}$ ) is dissolved in water a decomposition takes place according to the equation:



the products being calcium hydroxide and calcium sulphhydrate.

To determine the composition of solutions of calcium sulphid, three solutions were made as follows:

## CALCIUM SULPHID U.S.P. (MERCK).

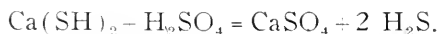
Solution I.	Solution II.	Solution III.
16 grs.	32 grs.	48 grs.
16 fl. oz.	16 fl. oz.	16 fl. oz.

In each case the calcium sulphid was boiled with the water, the solution cooled and filtered through paper, and placed in tightly stoppered pint bottles.

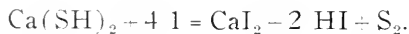
The following quantitative determinations were then made on each of these solutions:

1. 100 c.c. was titrated with  $\text{N}_{10} \text{H}_2\text{SO}_4$  to determine the alkalinity of the solutions. In this connection it should be borne in mind that only the alkalinity of the  $\text{Ca}(\text{OH})_2$  in solution is indicated by this titration. The  $\text{Ca}(\text{SH})_2$  is also decomposed by the acid, but for each equivalent of  $\text{H}_2\text{SO}_4$  added, an equivalent of  $\text{H}_2\text{S}$  is liberated, thus neutralizing the effect of the alkalinity of the  $\text{Ca}(\text{SH})_2$ .

The equation is:



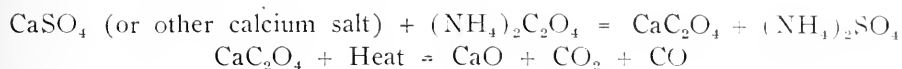
2. 50 c.c. was titrated with  $\text{N}_{10}$  iodine solution using starch solution as indicator. This indicates the amount of calcium sulphhydrate in solution according to the equation:



Instead of calculating these results to calcium sulphhydrate they may be calculated to the original amounts of calcium sulphid dissolved before their decomposition into  $\text{Ca}(\text{OH})_2$  and  $\text{Ca}(\text{SH})_2$ . See table below.

3. 25 c.c. were treated with a slight excess of ammonium oxalate in order to determine the total calcium (hydroxide, sulphhydrate and sulphate). Insoluble calcium oxalate is precipitated. This is collected on a filter, ignited at white heat and weighed as  $\text{CaO}$ . The reactions involved are:

\*I am indebted to Mr. H. W. Jones for this study in the chemistry of calcium sulphid.



Knowing the amounts of  $\text{Ca}(\text{OH})_2$  and  $\text{Ca}(\text{SH})_2$  in solution we may now calculate these in terms of  $\text{CaO}$ , deduct them from the total  $\text{CaO}$ , and calculate the balance as  $\text{CaSO}_4$ .

Following are the results obtained by these operations:

Solution	$\text{Ca}(\text{OH})_2$	$\text{Ca}(\text{SH})_2$	$\text{CaS}$	$\text{CaSO}_4$
I	.0467 gm. 0.213 gr.	.0674 gm. 0.308 gr.	.0916 gm. 0.418 gr.	.0777 gm. 0.23 gr.
II	.0636 gm. 0.291 gr.	.0922 gm. 0.421 gr.	.0125 gm. 0.572 gr.	.0103 gm. 0.47 gr.
III	.0644 gm. 0.294 gr.	.0922 gm. 0.421 gr.	.0125 gm. 0.572 gr.	.0131 gm. 0.598 gr.

In grams per 100 c.c., and grains per fl. oz.

These results show that the saturation point lies between 2 and 3 grains of calcium sulphid per fl. oz. In other words above 2 grs. per fl. oz. no more material is dissolved.

These solutions are perfectly clear and bright and show no trace of heavy metals when treated qualitatively.

Theoretically there are five possible sulphides of calcium, but only two of these are well-known: viz., the monosulphide ( $\text{CaS}$ ) and the pentasulphide ( $\text{CaS}_5$ ). The pentasulphide is obtained by heating milk of lime with sulphur.



This is what is known as Vleming's solution.

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# A PRELIMINARY MEDICAL STUDY OF THE POSITIVE CASES FOUND IN THE FIRST YEAR'S WORK OF THE MICHIGAN TUBERCULOSIS SURVEY

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THE nature and the methods of this survey have already been given in this Journal (Vol. 1, p. 134; Vol. 2, p. 206). Out of 11,528 persons examined, 2957 were diagnosed as positively tuberculous. These cases have been tabulated as shown in Table I, which represents the positive cases found in one of the smaller counties.

TABLE I

ALGER Co.																					
NO.	AGE	SEX	PNEUMONIA	PLEURISY	HEMORRHAGE	LOSS OF WEIGHT	ASSOCIATION		TEMP. PULSE			COUGH	EXPECTORATION	NIGHT-SWEATS	STAGE			LOCATION		RALES	CALMETTE
							MALE	FEMALE	A. M.	P. M.	PULSE				INCIDENT	MOD.	ADV.	RT.	LEFT		
1	43	M	-	-	+	+	-	-	-	+	-	+	+	-	+	+		U	U	-	+
2	21	M	-	-	-	-	-	-	-	-	-	-	-	-	+			U	U	+	-
3	54	F	-	-	+	-	B.	M <sup>3S</sup>	-	-	+	+	+	+	+			U	U	+	-
4	39	M	-	-	-	+	-	-	+	-	-	-	-	+	+	+		U	U	+	+
5	50	M	-	-	-	+	-	-	+	-	-	-	-	+	+	+		U	U	+	-
6	25	F	+	-	-	+	-	-	+	-	+	+	-	-	+			U	U	-	-
7	25	M	+	-	-	-	-	-	-	-	+	+	+	-	+	+		U	U	-	-
8	32	M	+	-	-	-	-	-	-	+	-	+	+	-	+	+		U	U	-	-
9	41	F	+	+	+	+	-	H	+	+	+	-	-	-	+	+		U	U	-	-
10	33	F	+	+	+	+	-	-	-	+	-	-	-	-	+	+		U	U	-	-
11	44	F	+	+	+	+	-	-	-	+	-	-	-	-	+	+		U	U	-	-
12	28	F	O	O	O	+	-	-	-	+	+	+	+	+	+	+		U	U	-	-
13	27	F	-	-	-	+	-	-	-	+	+	+	+	-	+			U	U	+	+
14	39	F	-	-	-	+	-	-	-	+	+	+	+	-	+	+		U	U	+	+
15	31	F	-	-	-	+	-	-	-	-	-	+	+	-	+	+		U	U	+	+
16	2	M	-	-	-	-	-	M	+	-	O	+	O	O	+	+		U	U	+	+
17	34	M	-	-	+	+	-	-	+	-	+	+	+	-	+	+		U	U	+	+
18	32	F	2	-	+	+	-	M	-	+	+	-	-	-	-	+		U	U	+	+
19	27	M	+	-	-	+	-	W	+	-	+	-	-	-	+	+		U	U	+	+
20	21	F	+	+	-	+	-	-	+	-	+	+	+	+	+	+		U	U	+	+
21	33	M	+	-	+	+	-	-	-	-	+	+	+	-	+	+		U	U	+	+
22	57	M	-	-	+	+	-	-	-	-	-	+	+	+	+	+		U	U	-	-
23	45	M	-	-	-	+	-	-	-	-	-	+	+	+	+	+		U	U	-	-
24	22	M	-	-	-	+	-	-	-	-	+	+	+	+	+	+		U	U	+	+
25	41	F	+	-	-	+	-	-	+	-	+	+	+	+	+	+		U	U	+	+
26	14	F	-	-	-	O	-	-	+	+	+	-	-	-	+	+		U	U	-	-
27	29	F	-	+	-	-	-	A	+	+	+	+	-	-	+	+		U	U	-	-
28	27	F	-	-	-	+	-	S	+	+	+	+	-	-	+	+		U	U	-	-
29	42	F	+	-	-	+	-	-	+	+	+	-	-	-	+	+		U	U	-	-
30	36	F	-	+	-	+	B	M <sup>2S</sup>	-	+	+	-	-	-	+	+		U	U	+	-
31	18	F	+	-	-	-	-	-	+	-	-	+	+	-	+	+		U	U	-	+
32	23	F	-	-	-	+	-	S	-	+	+	+	+	-	+	+		U	U	-	-
33	43	M	-	+	-	+	B	W	+	-	+	+	+	+	-	+		U	U	-	-
34	13	F	-	-	-	+	-	-	+	-	+	+	+	-	-	+		U	U	-	-
35	32	F	-	-	-	+	-	-	-	-	+	+	+	-	+	+		U	U	-	-
36	11	F	-	-	-	+	-	-	+	-	+	+	+	-	+	+		U	U	-	-
37	47	F	-	-	-	+	H	-	+	-	+	-	-	-	+	+		U	U	+	+
38	30	F	+	+	+	+	-	-	-	-	-	+	+	+	+	+		U	U	+	+
39	22	F	+	+	+	+	-	-	-	-	-	+	+	+	+	+		U	U	+	+
40	11	M	-	-	-	-	-	-	-	+	-	-	-	-	+	+		U	U	-	+

TABLE I (Cont'd).

ALGER Co.																					
NO.	AGE	SEX	PNEUMONIA	PLEURISY	HEMORRHAGE	LOSS OF WEIGHT	ASSOCIATION		TEMP. PULSE			COUGH	EXPECTORATION	NIGHT-SWEATS	STAGES			LOCATION		RALES	CALMETTE
							MALE	FEMALE	A. M.	P. M.	PULSE				INCIDENT	MOD.	ADV.	RT.	LEFT		
41	33	M	+	+	+	-	U	-	+	-	-	-	-	+	+	+	U	U	+	+	
42	6	M	-	-	-	-	F	-	-	-	-	-	-	-	+	+	U	U	-	-	
43	24	M	-	-	-	+	-	-	+	-	-	+	+	+	+	+	LU	U	+	-	
44	53	F	-	-	-	-	-	M	-	-	-	+	+	+	+	+	M	U	-	-	
45	38	F	-	-	-	-	-	-	-	-	-	+	+	-	-	+	U	UL	+	-	
46	24	M	-	+	-	-	-	-	+	+	+	+	+	-	-	+	UML	U	-	+	
47	28	F	-	-	-	+	F	-	-	-	+	+	+	-	-	+	ML	U	-	+	
48	46	M	-	-	-	-	-	-	-	-	+	+	+	-	-	+	UML	U	-	-	
49	19	M	-	+	-	-	-	-	-	-	-	-	-	-	-	+	U	U	-	A	
50	19	M	-	-	+	+	-	A	-	-	-	-	-	-	-	+	U	U	-	A	
51	19	F	-	-	-	+	B	-	-	-	+	-	-	-	-	+	U	U	-	A	
52	9	F	3	-	-	-	-	-	+	-	+	-	-	O	+	+	U	U	-	A	
53	26	F	-	-	-	-	-	M	-	-	-	-	-	-	+	+	U	U	-	AA	
54	11	F	-	-	-	-	-	M	-	-	-	-	-	-	+	+	Not given		-	-	

The above table is largely self explanatory. Under association it will be noted that a classification has been made according to sex, the initial letter being used in each instance to designate the member of the family afflicted. Thus, in the male column B, refers to brother, H, husband, F, father, S, son, and U, uncle; whereas in the female column, M represents mother, S, sister, W, wife and A, aunt. With regard to temperature, the symbol + has been used to designate any temperature occurring in an individual of fifteen years or over and which registers between 99 and 100 degrees F. In children no temperature below 99.6 was recorded as positive. Temperatures ranging from 100.1 to 101 inclusive are recorded as ++ and any temperature above 101 has been recorded as +++. With regard to pulse in individuals fifteen years or over a pulse ranging from 90 to 120 has been classified as +; between 120 and 130 ++ and above the latter point +++. Here again in children no pulse below 110 has been classified as positive. In connection with location of lesion initials have again been used to designate the lobe of the lung affected as U, upper; M, middle and L, lower lobe. In the last column are given not only the results with regard to the occurrence of positive and negative tuberculin reactions, but the letters A and P are used to designate cases which are apparently arrested and those showing physical signs indicative of pleurisy respectively.

## AGE, SEX, AND ASSOCIATION.

Among 2923 cases in which the sex was designated there were 1786 females and 1137 males. With regard to age, 764, or 26.1%, were fifteen years or less; 1041, or 35.6%, were between sixteen and thirty years inclusive; 747, or 25.5% between thirty-one and forty-five years inclusive; 282, or 9.6%, were between forty-six and sixty inclusive; and 89, or .3%, were between sixty-one and eighty years. Sixty-one and one-tenth per cent of all cases occurred between the ages of sixteen and forty-five.



Chart 1 gives the number of cases which gave a history of association with a single tuberculous member of the family. All cases in which two or more sources of infection occurred have been excluded. It will be noted that during the first fifteen years of life the source of infection in the majority

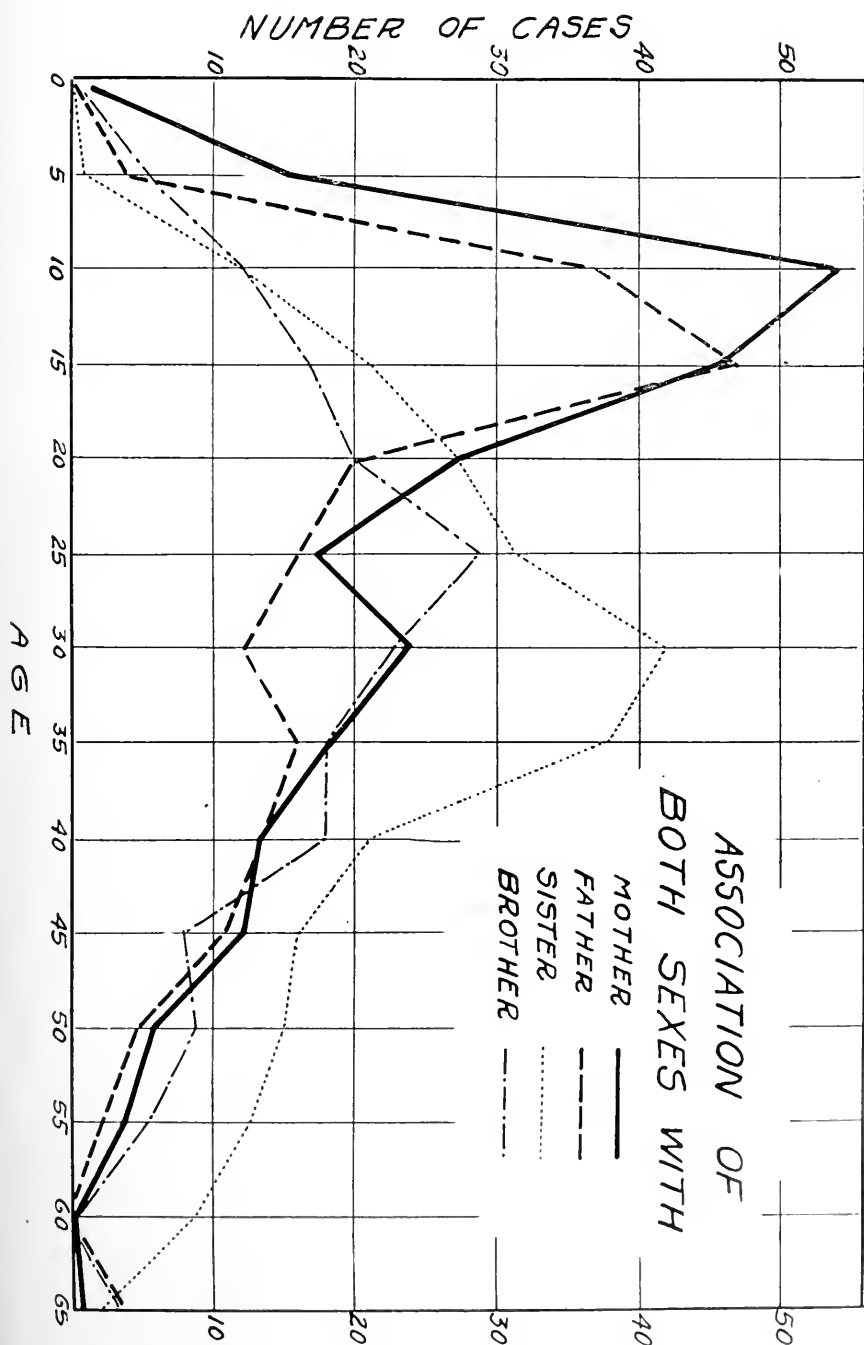


Chart 1.

of instances is either the father or mother, while beyond twenty years of age a history of tuberculosis in brother or sister predominates, the brother line reaching its maximum at twenty-five and the sister line at thirty years. The part played by intimate association is well illustrated in Charts 2 and 3.

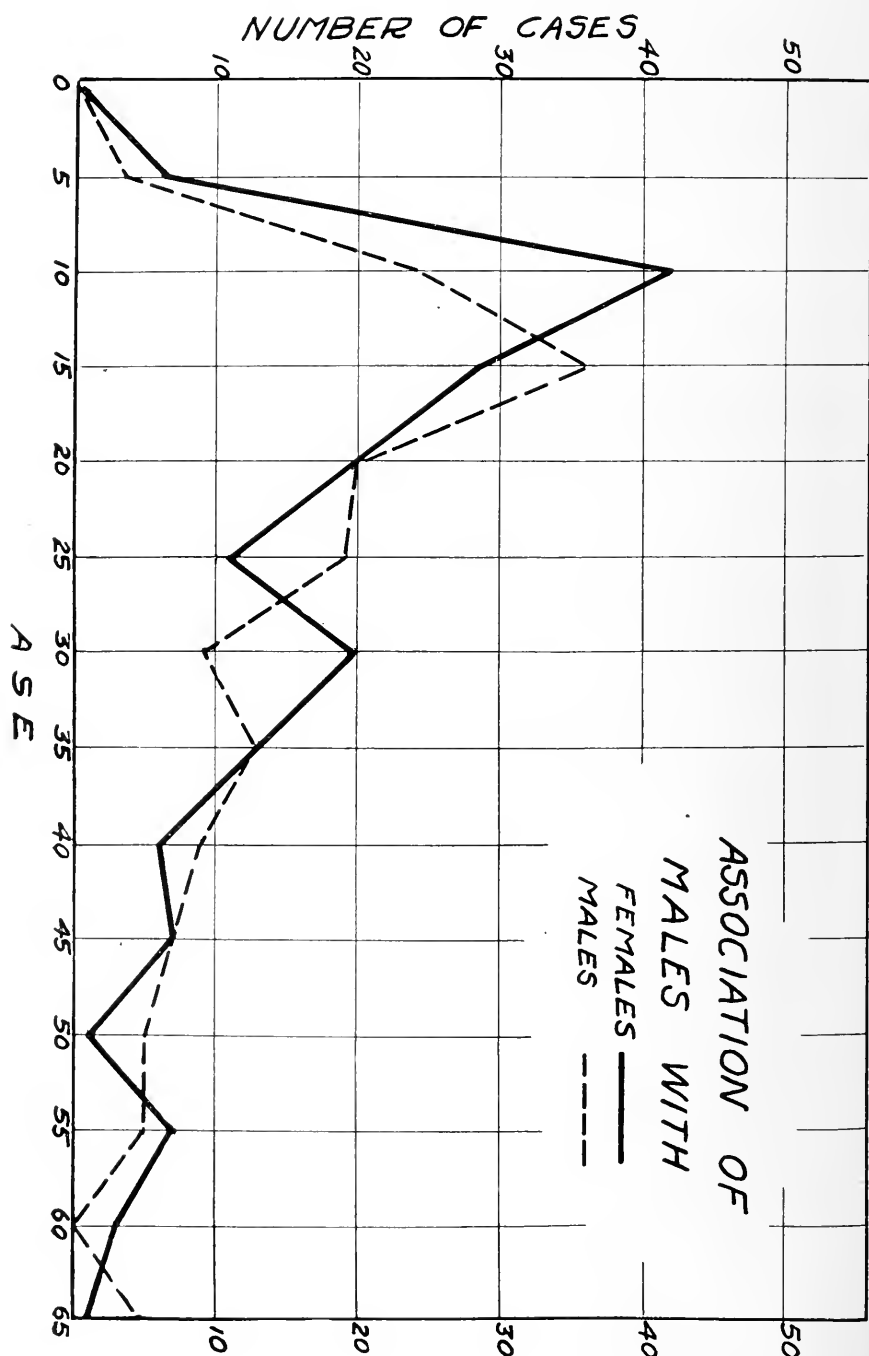


Chart 2.

Chart 2 represents the total number of males at the various ages giving a history of family association with a single tuberculous individual, while Chart 3 represents the total number of females at the various ages giving a history of family association with a single tuberculous individual. For obvious reasons

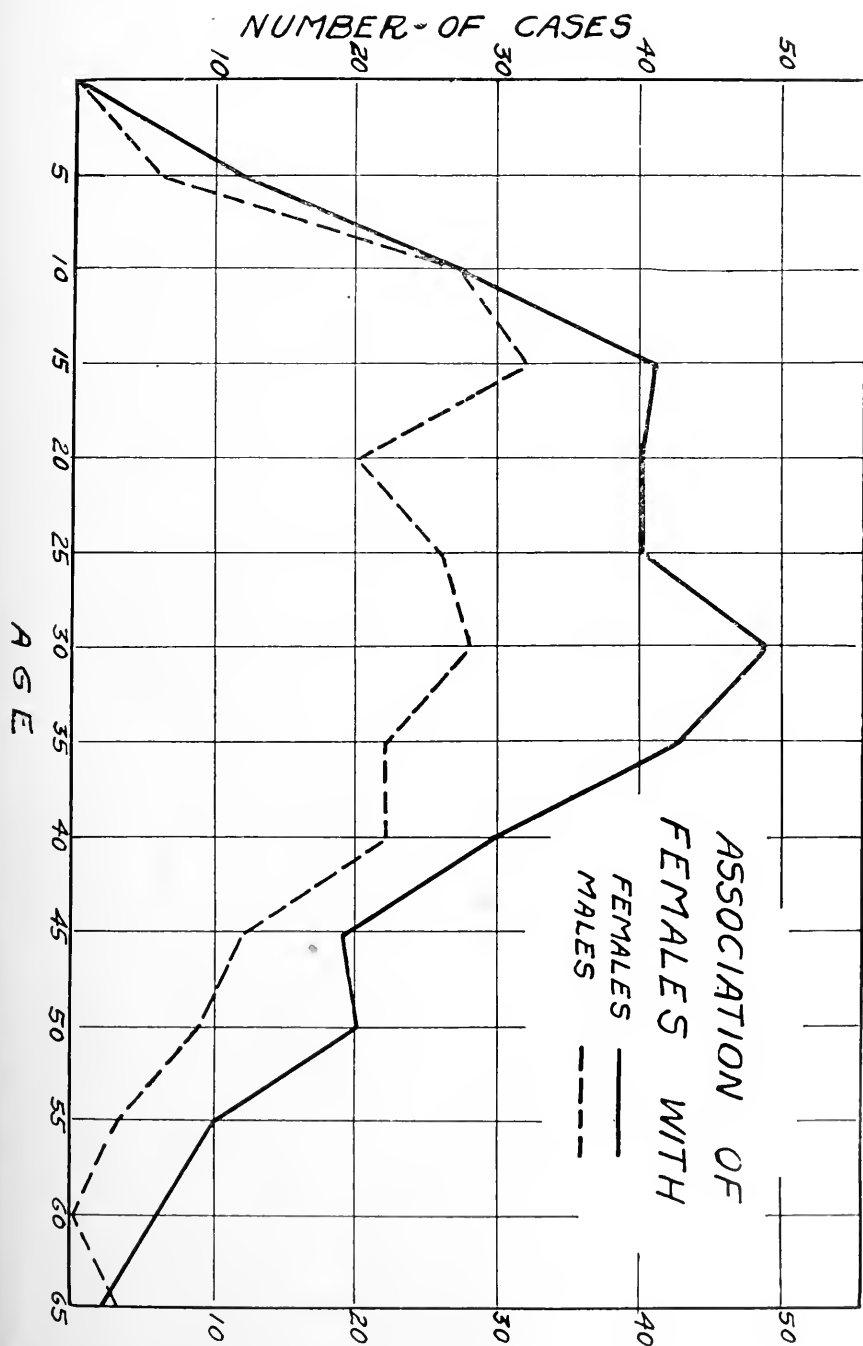


Chart 3.

individuals giving a history of tuberculosis in husband or wife have been omitted from these charts. In Chart 2 it will be noted that until ten years of age the female predominates as a source of infection, this being undoubtedly due to the close association existing between mother and child at this period of life, irrespective of difference in sex. From ten to fifteen years in boys the female line drops abruptly while the male becomes the greater source of infection. From fifteen on, the male source of infection generally predominates. In Chart 3, on the other hand, the female predominates throughout as a source of infection. It is interesting to note, however, that below the age of ten no marked difference in the two sexes is notable although here again the mother, as one would expect, represents the greater source of infection. After ten years the part played by intimate association between members of the same sex is most apparent.

#### PLEURISY.

Among the 2957 cases there were 605 who gave a definite history of pleuritic involvement at some time previous to, or coincident with, the examination, there being 46 cases in which no information with regard to this point was obtained. A history of pleuritic involvement was, therefore, obtained in 20.8% of all cases questioned with regard to this point. Owing to the definite character of pleuritic pain we believe that these statistics are most reliable. It is needless to state that all pleurisies associated with pneumonic attacks either as initial pleuritic involvement or as subsequently developing empyema were neglected.

#### HEMORRHAGE.

Among 2906 cases in which information with regard to the occurrence of pulmonary hemorrhage was obtained, 428, or 14.7%, had been afflicted at some time previous to the examination. Mere streaking of sputum was not considered, nor was the rusty sputum associated with pneumonic attacks. No case of a profuse pulmonary hemorrhage associated with a supposed pneumonic attack presented itself, although in this instance we would have been inclined to question the original diagnosis. Any amount of pure blood from a small clot to the expectoration of many ounces undoubtedly coming from the lower respiratory passages and accompanied by cough has been classified as pulmonary hemorrhage.

#### CLASSIFICATION BY STAGE.

The classification of cases of pulmonary trouble into various stages of the disease is always a difficult problem. We believe that such classification is of great practical importance for the information of the attending physician with regard to the ultimate prognosis, but we also feel that such a method necessarily partakes somewhat of the nature of scientific inaccuracy. The classification adopted by the National Association for the Study and Prevention of Tuberculosis has been followed as closely as possible and each individual case was classified as originally tabulated, the results obtained not being apparent until the final compilation of the complete table.

The cases have been divided into three stages as follows:

*First Stage.*

Incipient.

Slight or no constitutional symptoms (including particularly gastric or intestinal disturbance, or rapid loss of weight); slight or no elevation of temperature or acceleration of pulse at any time during the twenty-four hours.

Expectoration usually small in amount or absent.

Tubercle bacilli may be present or absent.

Slight infiltration limited to the apex of one or both lungs, or a small part of one lobe.

No tuberculous complication.

*Second Stage.*

Moderately advanced.

No marked impairment of function, either local or constitutional.

Marked infiltration more extensive than under incipient, with little or no evidence of cavity formation.

No serious tuberculous complications.

*Third Stage.*

Far advanced.

Marked impairment of function, local and constitutional.

Extensive localized infiltration or consolidation in one or more lobes.

Or serious tuberculous complications.

From Table II it will be seen that a classification of 2942 cases has been made according to stage. The interesting developments obtained from this classification are best demonstrated in Chart 4 which represents graphically the information obtained in table 2.

As can be seen from an examination of Chart 4 the symptoms not influenced by time of examination, as cough, expectoration, and night sweats, show practically the same percentage in the same stage at morning and afternoon clinics. From this fact we can fairly conclude that a definite degree of uniformity has been used in classification. It might be objected that the percentage of night sweats is high in the incipient cases and yet these for the most part represent cases showing but slight rise of temperature, no acceleration of the pulse, without expectoration and with physical signs limited to slight changes at one apex. Such cases cannot be classified otherwise than as incipient.

An examination of Chart 4 reveals at once most interesting variations between temperature and pulse findings in the incipient and moderately advanced cases. The most striking phenomenon is the higher percentage of cases showing a temperature between 99 and 100 degrees F. among the incipient as compared to the moderately advanced. This change, apparent even during the morning examinations, becomes pronounced during those of the afternoon. We believe that this is an indication of the fact that the true tuberculous temperature occurs most frequently in the incipient stage of the disease. It represents the rise in body temperature accompanying the specific reaction which occurs in the body of the recently infected tuberculous individual and which is frequently exhausted by the time the case becomes moderately advanced. Further evidence with regard to the fact that specific reaction occurs early in the disease is found in the results of tuberculin tests, which are conceded by all authorities to give positive reaction most frequently in the incipient stage and to disappear as the

CLASSIFICATION	TOTAL EXAMINED	POSITIVE TEMP.		POSITIVE PULSE		COUGH		EXPECTORATION		SWEATS	
		NUMBER	PERCENT	NUMBER	PERCENT	NUMBER	PERCENT	NUMBER	PERCENT	NUMBER	PERCENT
EXAMINED IN A.M.	747	334	44.7	316	42.3	382	51.2	258	34.5	134	17.9
	536	229	42.7	261	48.7	381	71.0	316	59.0	184	34.3
	89	37	41.6	65	61.8	81	91.0	78	87.6	46	51.7
EXAMINED IN P.M.	968	606	62.6	469	48.5	455	47.0	306	31.6	159	16.4
	524	295	56.3	283	54.0	382	72.9	303	57.8	174	33.3
	78	53	68.0	57	73.0	74	94.9	67	85.9	41	52.6

Table II.

case progresses. Thus in Chart 6, although the total number of cases is small, there is distinct evidence that the percentage of negative conjunctival reactions increases in direct proportion to the advancement of the disease process.

When we consider the pulse, we find that instead of paralleling the temperature, the exact reverse is true. Thus, whereas the greater percentage of slight

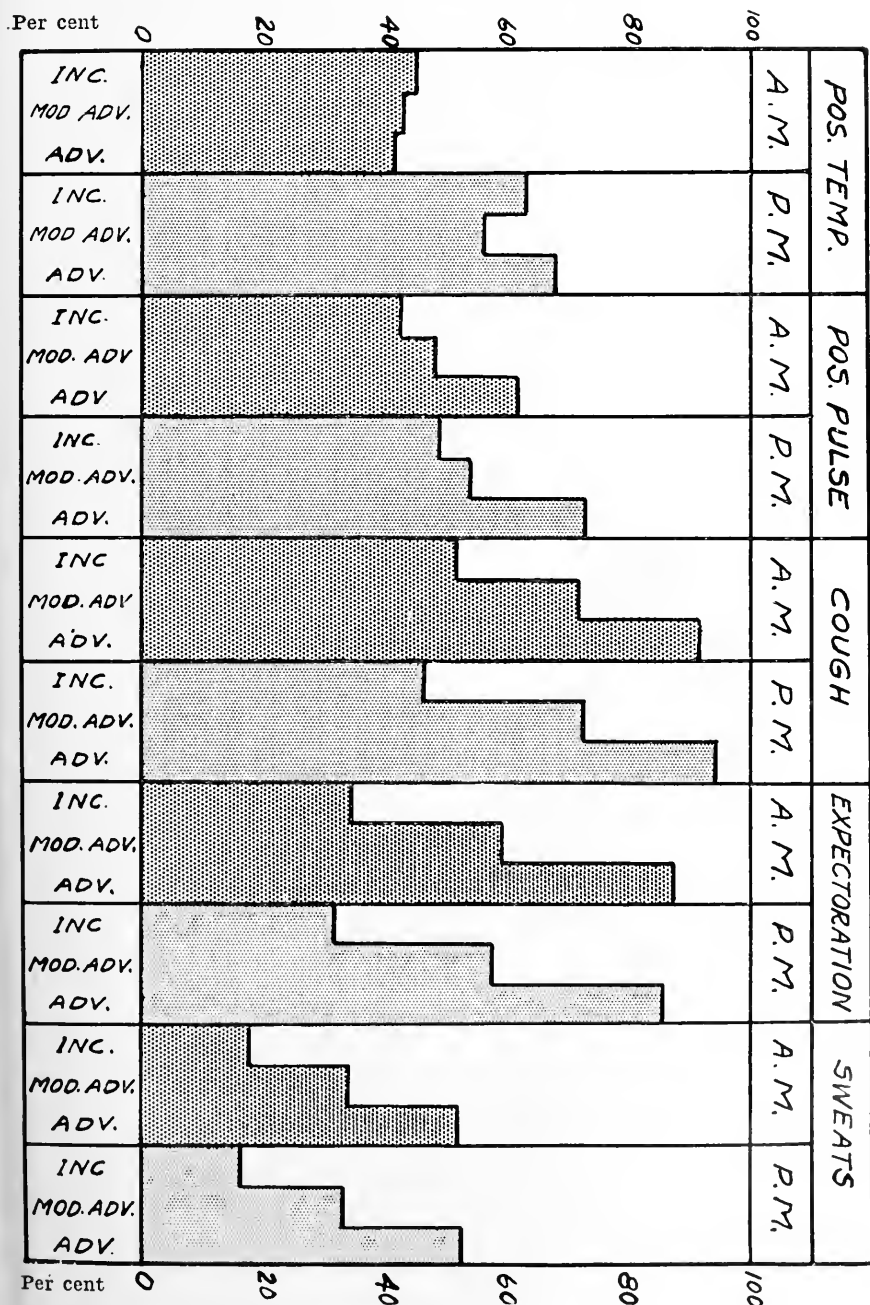


Chart 4.

febrile reactions occur in the early cases the percentage of high pulse is distinctly increased among the moderately advanced. The explanation of this discrepancy is probably to be found in the greater area of involvement and the relatively prolonged toxic action upon the myocardium in the moderately advanced cases.

#### EXTENT AND LOCATION OF PULMONARY INVOLVEMENT.

Of 2002 cases studied, the involvement was limited to one lobe in 990, or 49.5%; two lobes were affected in 717, or 35.8%; three in 212, or 10.6%; four in 57, or 3.8%; and involvement of all five lobes was recorded in 26, or 1.3%, of the cases.

From a study of Chart 5 it is evident that the right upper lobe is most frequently the site of disease, next in order come the left upper, the left lower, the right lower, and middle lobes. However, when we consider signs indicative of activity it becomes at once apparent that the left upper shows a higher per cent of activity than the right upper. Thus whereas râles were found associated with involvement of the right upper in only 45.6% of cases they were present in 57.2% of involvement of the left upper, in 58.2% of left lower involvement, in 60% of right lower, and in 62.4% of right middle involvement. Taking into consideration the larger number of cases of left lower involvement (483) as against those of right lower and middle, 310, and 284, respectively, we appear to have confirmation of the result obtained by Walsh who stated that in pulmonary tuberculosis the disease would seem to begin in most patients at the right apex, the subsequent extension being to the left apex and then to the base of the left lung.

#### TUBERCULIN TESTS.

The tuberculin tests employed by all examiners have consisted entirely of those dependent upon the local specific reaction occurring as the result of the application of tuberculin to the abraded surface of the skin or the mucous membrane of the conjunctiva. At first the majority of examiners hesitated to make use of the conjunctival test but finally, being convinced of its freedom from danger when properly applied in selected cases and its obvious superiority over the hypersensitive vaccination test, they have employed it freely. At many small places where clinics were held for a single day only, it was impossible to apply the test. However, in the larger centers where clinics were held on two or three successive days the test was extensively used on suspicious cases. The added value of the opportunity to examine the suspected individual on two successive days cannot be overestimated and frequently resulted in the obtaining of valuable positive evidence even in the presence of a negative tuberculin test. In no instance was a diagnosis made upon a positive tuberculin test alone, nor was a negative test interpreted as indicating the absence of tuberculous disease.

The record shows 751 tuberculous cases in which the results of the conjunctival test have been recorded, 583 of which were positive and 168 negative. Among these individuals, 113 gave a history of pleurisy at some time in the past and of this number 88, or 77.8%, responded positively to the test. There were 72 cases of pulmonary hemorrhage, 52 or 72.2% of which responded with a positive tuberculin test. Of the cases examined during the



afternoon clinic, 69.2% of the positive conjunctival tests showed a febrile reaction while this was obtained in only 55.2% of the tuberculous cases failing to respond to the test and examined at the same time of day.

In our preliminary report no tabulation of tuberculous conditions other

KEY:-

WITHOUT RALES

WITH RALES

LOCATION	NUMBER WITH RALES	PERCENT OF TOTAL INVOLVED	NUMBER WITHOUT RALES	PERCENT OF TOTAL INVOLVED	TOTAL
RIGHT UPPER	893	45.6	1064	54.4	1957
RIGHT MIDDLE	177	62.4	107	37.6	284
RIGHT LOWER	186	60.0	124	40.0	310
LEFT UPPER	691	57.2	520	42.8	1211
LEFT LOWER	281	58.2	202	41.8	483

PERCENT OF TOTAL INVOLVED

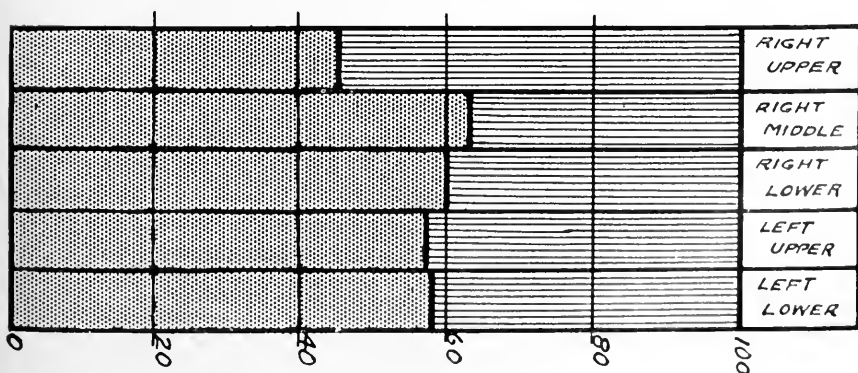


Chart 5.

than pulmonary has been presented. Cases of surgical tuberculosis without demonstrable pulmonary involvement have presented themselves for examination and cases in which the process was apparently limited to glandular involvement have been recorded. The total number of such cases is, however, comparatively

STAGE	POSITIVE CALMETTE	PERCENT OF TOTAL POS.	NEGATIVE CALMETTE	PERCENT OF TOTAL NEG.
INCIPIENT	425	72.9	107	63.6
MOD. ADV.	150	25.7	56	33.4
ADVANCED	8	1.4	5	3.0
TOTAL	583	100.0	168	100.0

AFTERNOON TEMPERATURE	POSITIVE	NEGATIVE	TOTAL	PERCENT POSITIVE	PERCENT NEGATIVE
POSITIVE CALMETTE	224	100	324	69.2	30.8
NEGATIVE CALMETTE	48	39	87	55.2	44.8

HISTORY OF	CALMETTE POSITIVE	CALMETTE NEGATIVE	TOTAL	PERCENT POSITIVE	PERCENT NEGATIVE
HEMIGE.	52	20	72	72.2	27.8
PLEURISY	88	25	113	77.8	22.2

KEY:-



POS. CALMETTE



NEG. CALMETTE

PERCENT OF TOTAL INVOLVED

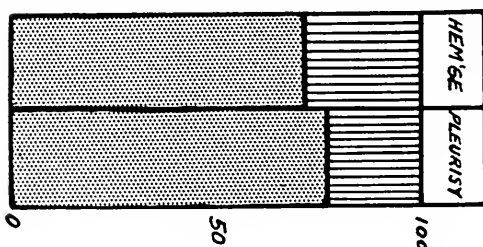


Chart 6.

small at present and would not appear to justify tabulation at this time. In the complete report it is our intention to give due consideration to the above mentioned types of the disease.

That the diagnosis of incipient cases particularly has been determined with great care is evidenced from the fact that of the 2957 cases tabulated we have definite records of 751 who presented themselves at the clinic for a second examination. No single symptom or test was relied upon as definitely determining the diagnosis, but the weight of total evidence eventually decided the result. That the work has been well done is best indicated by the results obtained from the total compilation with regard to various phases of the problem, which in no way varies from the logical or the expected. Whenever a doubt has remained in the mind of the examiner, the case has not been pronounced positive, but has been classified as suspicious. In all positive and suspicious cases a copy of the physical findings has been placed in the hands of the attending physician and the patient has been advised to call upon his physician for periodic examinations and medical supervision. Thus in the case of a given individual classified as suspicious, the findings of the examiner consisting of prolonged expiration at the right apex associated with a suggestion of crepitant râles beneath the outer third of the right clavicle are furnished the attending physician who by subsequent examination of the chest, repeated if necessary, will eventually determine the tuberculous or nontuberculous character of the case. It is safe to infer that individuals, who have availed themselves of the opportunities afforded by the clinic, will in the majority of instances see that they have proper subsequent supervision.

Credit is due the medical profession of the state for their hearty cooperation in the work of the survey. Physicians generally showed great interest, attended the clinics and aided materially in the discovery of cases in their locality.

In conclusion we wish again to emphasize the fact that as a result of the survey, 2957 positive cases have already been discovered. These represent an equal number of potential sources of infection to others, some actively infectious at present; 1715 incipient cases, mostly closed, innocuous at present but representing sources of infection in the future provided they are not properly cared for. The great majority of these 1715 cases will, under proper supervision, completely recover without at any time being sources of danger to others and ultimately will take their places among the useful citizens of the commonwealth.

# SPIROCHÆTES\*

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## PART II.

### TRANSMISSION OF SPIRONEMA AND TREPONEMA TO MAN AND ANIMALS.

UNDER natural conditions the transmission of a blood-inhabiting *Spironema* to man or animals is effected through the bite of an infected blood-sucking insect. The transmitter in each instance is highly, if not strictly, specific, although other blood-sucking insects may also be infected by sucking the blood of an animal which is suffering from an infection with any of the pathogenic blood spirone-mata. These unnaturally infected ticks, bedbugs, fleas, or lice are not good transmitting agents as compared with the natural carrier of the infection. That the *Spironema* in such nonspecific insects can survive for some time can be shown when the disease is produced in a susceptible animal by inoculating it with the crushed material of the infected insects. It is possible, therefore, that an infection can be occasioned by smearing the excreta or crushed body contents of the infected insect over any defect of the epidermic layer of a susceptible subject. For example, in the case of *Spironema recurrentis*, both body lice and bedbugs may be infected by sucking the blood of a patient suffering from the European relapsing fever, but the lice alone can transmit the disease to the next person they bite. Bedbugs are never known to spread the infection by their bites although by crushing the infected bugs directly over a minute skin trauma (scratch, etc.) a person may become infected. A brief summary is given below of the natural intermediary hosts of different bearing spirone-mata, as well as certain experimental data bearing on the role of other blood-sucking insects and on the susceptibility of various animals of each *Spironema*.

*Spironema recurrentis*, the causative agent of the European relapsing fever, is naturally transmitted by *Pediculus corporis*. *Pediculus capiti* was found by Gonder to be incapable of transmitting the disease, although its body may contain the organisms. The common bedbugs (*Acanthia lectularia*) may likewise harbor the *Spironema* for as long<sup>130, 131, 132</sup> as sixty days, but according to the experiments of various investigators, does not spread the infection. The rat louse (*Hematopinus spirulosis*) can carry the infection from rat to rat, while the monkey louse does the same among monkeys. Breinl and Kinghorn,<sup>133</sup> as well as Neumann,<sup>134</sup> Manteufel,<sup>135</sup> and Sergeant and Foley<sup>136</sup> succeeded in transmitting the infection to rats with ticks (*Ornithodoros moubata*). Schuberg and Kuhn report a successful transmission by *Stomoxys*, the blood-sucking flies.

The infection can be transmitted subcutaneously as well as *per os* in experimental animals.

Infected organs fed to rats produce the infection in these animals, as shown by Uhlenhuth and Haendel,<sup>137</sup> and others. Manteufel considers the uninjured

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Harvey lecture delivered on February 5, 1916, at the New York Academy of Medicine.

skin permeable to *S. recurrenti*, and Nattan-Larrier produced the infection *per vagina*, *per penis*, etc., in rats, while Gozony successfully transmitted the disease also by means of subcutaneous, conjunctival, and intestinal application of the Spironema. Schellack,<sup>138</sup> who obtained a positive result in one out of twenty-eight experiments on rats, was able to demonstrate a microscopical defect of the skin at the point of entrance. The organism is experimentally transmissible to monkeys, and from monkeys to rats, mice, guinea pigs, and sometimes rabbits.

*S. duttoni*, the causative agent of the African tick fever, is normally carried by *Ornithodoros moubata*, as was recognized by Dutton and Todd,<sup>139</sup> and Koch.<sup>139</sup> The last named investigator discovered the Spironema in the ovaries four to five days after the tick had sucked the infected blood. Carter<sup>140</sup> confirmed this finding, while Neumann<sup>134</sup> found the organisms in freshly laid eggs. Hereditary infection for one or more generations was shown to occur by the study of Dutton and Todd, Wolbach,<sup>141</sup> and others. The tick is infectious an hour after sucking and remains so as long as 90 days (Wittrock<sup>142</sup>). This author always found the Spironema in the infective ticks. *Ornithodoros savignyi* has been suspected of carrying the infection,<sup>143</sup> and Brumpt once succeeded in infecting a monkey by this tick. Robledo holds *Argas americanus* responsible for the spreading of *S. nozvi* (the American type of relapsing fever) in Columbia, but this theory calls for further investigation. According to Breinl and Kinghorn,<sup>144</sup> the rat's fetus may be infected through the placenta when a mother rat is inoculated with *S. duttoni*. The organism is experimentally transmissible to rats, mice, monkeys, guinea pigs, and rarely to rabbits.

As has been briefly mentioned elsewhere, Leishman, Fantham, Hindle, and others assume a granular or coccoid phase in the life history of this and allied species, and maintain that the Spironema gradually undergoes granulation when it reaches the tick's body and multiplies in the Malpighian tubules and ovaries. The tick becomes infective after an incubation of 1 to 2 days at 37°C. Hindle<sup>70</sup> demonstrated the infectivity of the coxal fluid in which he found numerous granules and some spironemata. This investigator thinks that the Spironema or infective granules in the coxal fluid enter the body of persons through the wound produced by the bite of the tick. The infected eggs become infective after being incubated, as was demonstrated by Hindle by injecting the crushed material into the susceptible animals; Leishman<sup>145, 146</sup> found numerous spiral rods in the infected tick eggs when the latter were incubated for a few days at 35°C. Schubert and Manteufel showed the infectivity of the ticks to be lost when they are kept at a temperature below 22° C., but Gonder failed to find any such difference. Marchoux and Couvy, Gleitmann, Wolbach, Wittrock, Kleine and Eckard, and others, believe that, wherever infectivity is present, there are always to be found some typical spironemata, either in the tick or in its eggs.

*Spironema berbera* (the North African type) is carried by *Pediculus corporis*, but not by *Argas*, the flea or bedbug<sup>147, 148</sup> (Sergeant, Gillet, and Foley). *S. carteri* is also transmitted by body lice. In this case Mackie<sup>149</sup> found the organisms more numerous in the female lice than in the male; they are distributed in the mouth, stomach, and digestive tract. Mackie believes, however, that *Acanthia lectularia* sometimes carries the infection.

Among the ticks which transmit *Spironema* in cattle and sheep may be mentioned *Boophilus decoloratus* and *Rhipicephalus cvevtsi*. The virus is carried by heredity. The causative agent of chicken fever, *S. gallinarum*, is carried by *Argas persicus* under natural conditions, while other species (*Argas reflexus* and *Argas miniatus*) can transmit the disease experimentally.<sup>135</sup> *Ornithodoros moubata* is doubtful, as Schellack<sup>138</sup> failed to produce the infection while Fülleborn and Mayer<sup>150</sup> claim a success with this organism. Schellack was able to produce the infection in 3 out of the 15 experiments performed by him on chickens by the percutaneous application of the infected blood. Feeding fowls with infected ticks may cause the infection. The organism is experimentally transmissible to ducks, geese, sparrows, canaries, and sometimes rabbits. *S. icterohemorrhagiæ*, the causative organism of Weil's disease, has been shown by Inada and his associates to be but rarely conveyed by direct contact, but no natural intermediary hosts have been discovered. The organisms are abundantly present in the urine during the convalescent stage and they are fully virulent for guinea pigs. According to the experiments of Inada and his associates, the *Spironema* as contained in the liver emulsion is capable of penetrating an apparently uninjured skin of the guinea pig a short time after contact (5 minutes is sufficient to cause infection).<sup>\*</sup> Therefore, it is altogether possible to infect a person through direct contact with some of the excreta of a patient. The infection can be induced in guinea pigs by introducing the infected material into the stomach after it has been previously neutralized with bicarbonate of soda. In regard to the *Spironema* found by Futaki and others at the site of or in glands adjacent to the rat bite, we must assume that this represents the occurrence in rats of a pathogenic *Spironema* which produces fever and other symptoms when transmitted to human subjects. Further investigation in this direction is most desirable. The organism is most easily experimentally transmissible to guinea pigs. Monkeys, rats, and mice are less susceptible.

Prior to Futaki's work there appeared a report by Kitagawa and Mukoyama, who also found a *spironema* in the inflamed tissue of the bitten finger of a woman. By transmitting the tissue into guinea pigs and white rats, these authors claim to have reproduced symptoms resembling the so-called rat-bite fever. In the smears of the kidney and liver taken from the dead animals, they found two types of spiral organisms; namely, in the guinea pig tissues, the refringens type; and in the white rat, the minute and short type. In examining the preparations kindly sent to me by the authors, I found their findings to be entirely correct, but the refringens type is more like *T. macrodentium* and a large number of bacteria, such as fusiform bacilli, and big rods, etc., were also present in the same preparations. As to the short type, one can only say that its morphology is almost indistinguishable from that of *S. muris* or *S. microgyratum*. These organisms do not agree with the illustrations and description of the *Spironema* reported some time later by Futaki and others. In the case of Kitagawa and Mukoyama, the local and general symptoms may have been due to a mixed infection by the oral flora of a rat.

The *Spironema* of relapsing and tick fevers also cause in man a well char-

<sup>\*</sup>Of eight guinea pigs experimented upon, only one escaped the infection which developed in from ten to twelve days with typical symptoms.

acterized type of fever accompanied by two attacks interrupted by a period of apyrexia lasting several days. During the apyrexia the blood is free of the parasites. But it is not at all rare for the recidive to be repeated more than once. While at the highest point of the fever the organisms are most abundant in the blood, they are also present in different organs. Dutton and Todd, Breinl, Leishman, and Fantham believe that the spironemata are taken up by phagocytes within which they undergo transformation into the granular phase which in turn gives rise to the new generation of spironema. Balfour considered the intraglobular forms of *Spironema granulosa penetrans* (similar to, probably identical with *S. gallinarum*) as asexual, and the extracellular forms as a sexual phase. Fantham observed some extracellular granules which may start the relapse. Darling would hold the phagocytized spironemata within the endothelial cells of the liver responsible for the source of the recidive, as he found the organism to remain intact for some time during convalescence. Grabitschewsky sees in the surviving resistant specimens, which had been shielded from destruction in various organs, the progeny of the organisms producing the second attack. In the case of *S. carteri*, Mackie assumed the possible existence of an ultramicroscopic phase, as the serum taken from a patient at the apyrexia period is said to be infective in spite of the absence of any spiral forms. It may be remarked that to detect a sparse number of any Spironemata under the microscope is one of the most difficult tasks, and one is very liable to overlook the organism.

*T. pallidum* and *T. pertenue* are the only pathogenic varieties among the group. In the case of syphilis our knowledge is quite complete, so far as the mode of transmission is concerned. On the other hand, much still remains to be learned regarding the manner in which yaws is communicated from person to person. Probably, like syphilis, its infection is spread by direct contact with a patient or any object which, after having been in contact with a patient, harbors the live organisms, although the possibility of transmission through flies, mosquitoes and ticks is not excluded. Castellani and Chalmers<sup>151</sup> quote an instance in which a fly which sucked on a yaws papule infected a monkey whose eyebrow was scarified. Modder<sup>152</sup> assumes transmission of yaws by ticks (*Argas* and *Ixodes*) in Ceylon. It is said that vaccination and wet nursing spread the infection. In order to facilitate their entrance into the human body both organisms need only a microscopical defect of the epidermis.

After penetrating the skin or mucous membrane, *T. pallidum* elicits a local reaction characterized by the circumscribed round cell infiltration known as chancre (primary lesion), then several weeks later, about the time when the chancre recedes, it proceeds to enter the adjacent lymph glands and general cutaneous and mucous membrane tissues, producing roseola, papules, and flat condyloma. At this period the organisms invade almost every tissue, producing the so-called secondary symptoms. Periostitis, meningitis, iritis, and laryngitis are very frequently observed. One of the most constant symptoms is the Wassermann reaction in the blood serum.

After a period of several months longer, during which the secondary manifestations abate, another period known as the tertiary stage may supervene, accompanied by still deeper tissue destruction caused in the organisms than at any

previous stage. It affects skin, bones, visceral organs, cardiovascular system, and central nervous system. The disease may be progressive or marked with alternate activity and latency. Yet in the latent period repeated abortions may occur. From the time of infection until the central nervous system is affected (general paralysis, tabes), the average period of latency of the disease is from eight to twelve years. During the tertiary stage the lesions are often gummatous and affect the connective tissue muscles and blood vessels, while in cases of general paralysis and tabes the parasites diffusely pervade the parenchyma.<sup>153, 154, 155, 156.</sup> This form is a syphilitic parenchymatous encephalomyelitis. In acquired syphilis *T. pallidum* has been demonstrated in every syphilitic condition. It was first demonstrated in the primary and secondary lesions by its discoverers, Schaudinn and Hoffmann; in liver gumma, by Schaudinn; in aortitis, by Wright and Richardson;<sup>157</sup> Schmorl,<sup>158</sup> and Reuter;<sup>159</sup> in arteritis cerebri, by Bender;<sup>160</sup> in heart muscles and pancreatitis, by Warthin;<sup>161</sup> in adrenal glands, by Hoffmann,<sup>162</sup> Jacquet and Sézary;<sup>163</sup> in nephritis, by Hoffmann;<sup>164</sup> in the cerebrospinal fluid, by Hoffmann,<sup>165</sup> Nichols and Hough,<sup>166</sup> and Sézary and Paillard;<sup>167</sup> in the blood during the secondary stage, by Uhlenhuth and Mulzer;<sup>168</sup> in paresis, by Graves;<sup>169</sup> in interstitial keratitis, by Igersheimer;<sup>170</sup> in cerebral gumma, by Dunlap;<sup>171</sup> in the parietic brains, by Noguchi and Moore,<sup>172</sup> Marinesco and Minae,<sup>173</sup> Levaditi, Marie and Bankowsky,<sup>174</sup> Mott, Rosanoff,<sup>175</sup> Tomaszewski and Foster,<sup>176</sup> Wile,<sup>177</sup> and others; in spinal cord, by Noguchi,<sup>178</sup> Versé,<sup>179</sup> and others. It should be mentioned that the first demonstration of *T. pallidum* in sections of tissues from acquired syphilis was accomplished by Bertarelli and Volpino<sup>180</sup> by means of their silver impregnation method which has since been superseded by a similar procedure amended by Levaditi.

In congenital syphilis the number of organisms present in the different organs and in different fetuses varies greatly. In some it may be extremely tedious to demonstrate the organisms, in others the whole fetus may be thickly interwoven with the intertwining nets of treponemata. The favorite site of invasion is the liver and skin, although stomach, intestines, adrenals, kidney, spleen, heart muscles, pancreas, bone marrow, lymph glands, thymus, testes, ovaries, and brain have been shown to contain the parasites, even in large numbers in certain instances.<sup>181</sup> The placenta and navel cord are also affected. For the first demonstration of the organisms in congenital lues, we are indebted to Levaditi,<sup>182</sup> who introduced his well known silver impregnation method for this study. According to personal experiences in connection with syphilitic infants who lived several days after birth, the number of pallida present was always very small and it sometimes required many hours' search to find a single specimen. A striking difference between syphilis and yaws is the absence in yaws of visceral affections and of the nervous involvement. Much yet remains to be investigated with regard to the relationship between syphilis and yaws, the causative agents of which bear so great a morphological, and, to a certain extent, a biological resemblance toward each other.

The transmissibility of syphilis to animals was long the subject of study by earlier investigators, but the first conclusive experiments in this connection were furnished by Metchnikoff and Roux<sup>183</sup> who succeeded before the discovery of



*T. pallidum* by Schaudinn in producing the primary and secondary lesions in the chimpanzee. It was also shown that these lesions were transferable to further series of animals. Immediately after the discovery of *T. pallidum* in human syphilitic tissues, Metchnikoff and Roux found the same organism in experimental syphilis, thus closing up the first link of the chain of evidence which was to prove the specificity of the organism for syphilis. They also infected macacus monkeys with the virus derived from the chimpanzee. Soon afterward Schultze<sup>184</sup> and Bertarelli<sup>185</sup> produced syphilitic keratitis in rabbits, while Parodi<sup>186</sup> selected the testes (intratesticular) to transmit the human strain to the rabbit. This work has been extended and elaborated by later investigators, particularly by Neisser,<sup>187</sup> Hoffmann, Loehe, and Mulzer,<sup>188</sup> Uhlenhuth and Mulzer,<sup>189</sup> Grouven,<sup>190, 191, 192</sup> Nichols,<sup>193</sup> Tomaszewski,<sup>194, 195, 196, 197</sup> and others. In monkeys the best site for inoculation is the eyebrow, while in rabbits intratesticular, scrotal, intraocular and intracardial inoculations were recommended. For the purpose of keeping up the pallidum strain, the intratesticular mode is preferable, especially when it is desired to obtain a pure material for cultivation (Uhlenhuth, Noguchi); but in case of utilizing the lesions in order to determine the effect of a therapeutic agent, Hata<sup>128</sup> recommends the scrotal chancre method introduced by Tomaszewski,<sup>194</sup> wherein he is supported by the experience of Brown and Pearce.<sup>198</sup> With the purpose of causing a generalized syphilis in the rabbit—which animal is usually refractory to the systemic pallidum infection—Grouven reports the intracardial introduction of a large quantity of the pallidum in half-grown rabbits. My own numerous attempts to produce generalized syphilis by this method completely failed, probably owing to the difference in the strains employed. It may be mentioned, however, that with certain pallidum strains symptoms similar to human secondaries or tertiaries could be produced by means of intravenous or intratesticular inoculation. I have a few times observed iritis, keratitis, and squamous or ulcerative skin lesions, in the last of which the pallidum could be demonstrated. Nichols and Hough<sup>166</sup> were the first, however, to isolate a strain from a case of nervous recidive which constantly invaded the cornea, even before the local symptoms (testis) commenced to appear. This strain has most persistently caused keratitis and choroidoretinitis in rabbits. This phenomenon led Nichols to assume that the strain possessed a highly invasive character.<sup>199</sup> The patient from whom this strain was obtained died several months later of a rapidly progressive form of meningo-encephalitis, and the duration of the infection (from the time of the chancre to death) was very short. Nichols, therefore, considered that this case was explained by the character of the strain. Reasoner<sup>200</sup> also obtained a strain from a rapidly fatal case which was characterized by the early production of choroiditis in rabbits. In some rabbits the choroiditis was the only symptom in spite of its being introduced into the testis or vein. While studying ten different strains of *Treponema pallidum* I was once struck with the constancy with which the various types were associated with certain distinct characters of the lesions produced in rabbits. For example, I could discern the differences among different strains in the width, length, and number of curves to a given space, etc. I divided these strains into a thick, a thin, and a medium type. The differences were great enough to enable me to

identify different strains as belonging to any one of the three types.<sup>201</sup> In a series of passages covering a period of about one year and a half, it was found that the thin type produced a soft, diffusely swelling orchitis within 10 to 14 days and did not form any definite nodules even after six weeks. On the other hand, the thick type produced a hard, circumscribed nodule of varying size within about six weeks. Its development was unusually slow and the nodule remained for several more weeks. The character of the syphiloma produced by the medium type was a large, moderately firm orchitis, which started to be palpable at the end of about four weeks. As will be mentioned later, these three type strains were cultivated in an artificial medium and were found to retain their morphological characteristics unchanged. Again, my experience with the two parietic strains of *Treponema pallidum* transmitted from human brains to rabbits' testicles (using 36 rabbits for six specimens of brains) showed me that they were of lower virulence than the ordinary chancre strains in my possession, as they required 97 and 102 days respectively before the lesions could be definitely demonstrated.<sup>119</sup> With the usual skin strains four weeks' incubation is the average. Wile<sup>177</sup> recently reported a successful transmission of the pallidum from the living parietics to rabbits' testicles (using one rabbit for six specimens of brains) in which the lesions appeared within 14 days, and he concluded that the parietic strains were more virulent than the ordinary strains. It may be recalled that the persistent endeavors to produce syphilitic orchitis in rabbits by means of the parietic brains was not limited to a few investigators. Tomaszewski and Forster,<sup>202</sup> who in 1913 performed the Neisser-Pollack puncture on 62 cases at the University Institute in Berlin and found numerous examples of the motile pallida in 29 cases of the removed material, inoculated a large number of rabbits. Their results were uniformly negative. Marie, Levaditi and Bankowsky, Marinesco, Mott, and others also failed to obtain a single positive result. Another interesting feature characteristic of the strain obtained by Wile<sup>203</sup> is the readiness with which it at once adapted itself to an artificial culture medium, generally known to be unsuitable for the purpose of obtaining an initial growth with any strain which is transmitted to the rabbits' testicle. As I have pointed out on several previous occasions, a solid medium consisting of fresh tissue, ascitic fluid and agar, is not suitable for such a purpose, and this fact has been confirmed by numerous investigators (Uhlenhuth, Zinsser and Hopkins, and others).

It will be incomplete if we pass on without reviewing the interesting observations of Graves,<sup>169</sup> who succeeded in infecting a certain number of rabbits by injecting the blood of parietic patients. Graves obtained the blood in small glass ampules (sterile) which were immediately sealed. The different specimens were put in an incubator at about 37°C., and after a number of days the contents of these ampules were inoculated into the testicles of rabbits. Although the majority of the inoculations were negative, he found a strain developing in one of the animals. Morphologically, the organisms were the typical pallidum and produced local as well as generalized reactions (ulcerative lesions near the nostrils, anus, prepuce, vagina, etc.) wherein the organisms were demonstrated. The incubation period of average duration is about three to four weeks. This strain was characterized by the early appearance of keratitis in rabbits. The observation of

Graves furnishes us with a problem, viz., the fact that the sample of paretic blood sealed in a tube and left many weeks and months at an indifferent temperature was still capable of infecting a rabbit with such extreme severity. Yet Graves never succeeded in cultivating any strain of such samples; neither could he demonstrate the presence of any definite pallidum. Therefore, as Graves seems to think, *Treponema pallidum* must possess a stage of its life cycle which is still little understood by us. Can there be a resistant form which remains dormant for years until favorable conditions are secured? Clinical evidence has suggested this idea to certain syphilologists. Personal experiences with cultivated strains of *Treponema pallidum* do not justify my assuming the existence of a resistant form, except for the fact that the pallidum under cultural conditions is one of the most viable organisms. In suitable media it survives over one year when kept at 37°C., and it is not impossible that under naturally favorable conditions it may remain dormant for many years.

Several other animals besides monkeys and rabbits are susceptible of the disease. In dogs and sheep (Bertarelli, Hoffmann, Brüning), in guinea pigs and goats (Bertarelli), and in cats (Levaditi and Yamanouchi) specific keratitis has been produced. Testicles of guinea pigs (Truffi, Tomaszewski, W. H. Hoffmann, Uhlenhuth and Mulzer) and goats (Uhlenhuth and Mulzer) are also susceptible to infection by *Treponema pallidum*. Schereschewsky reported a scrotal chancre experimentally produced in a pig.

*Treponema pertenue* has been successfully transmitted into monkeys by Neisser, Baermann and Halberstädter<sup>204</sup> with skin papules, and by Castellani<sup>205</sup> with a punctate of the spleen of a patient. Nichols<sup>206</sup> transmitted it from man to *Macacus rhesus* and then from the latter to the rabbit. In the rabbit's testicle it produces a hard induration much like a syphilitic chancre. The parenchymatous orchitis finally extends over to the tunica and scrotum in which an extensive ulcerative indurated lesion results. When inoculated to the eyebrows of *Macacus rhesus*, the yaws organism produces highly destructive ulcerative papules which may remain unhealed for many months. In these lesions *Treponema pertenue* can easily be demonstrated. Halberstädter<sup>207</sup> observed a generalized eruption in an orang-outang 4 months after inoculation. In lower monkeys the lesion remains localized and heals in from three to thirteen weeks; sometimes it may result in a serpiginous recidive which tends to become diffuse.

#### FILTERABILITY OF SPIRONEMA AND TREPONEMA.

According to the experiments of Novy and Knapp,<sup>18</sup> *Spiroplasma recurrentis* and *S. duttoni* pass in one form or another through the pores of Berkefeld filters, the walls of which were either previously shaved off to a thickness of 1.4 to 2.5 mm. or left intact (4.2 mm.) as the filtrates obtained by this procedure were able to produce in susceptible animals a slight infection accompanied by sparse spirochetes appearing in the blood. The scarcity of the organism is ascribed to the presence of immune substances in the filtrate which was simultaneously introduced. Breinl and Kinghorn<sup>133</sup> obtained similar results with unmodified filters. In neither instance did the infective filtrates contain the Spiroplasma in its spiral form. Their experiments tended to suggest a filterable phase in the life cycle of

this organism. Todd and Wolbach<sup>208</sup> report the successful filtration of the organism through the Berkefeld filters N and V, by pressures of fifty to ninety pounds to the square inch. Under these conditions the organism traversed the tortuous pores of the filters and was seen to have retained its usual spiral form when it appeared in the filtrate. Todd succeeded in finding the organisms in the filtrates of one experiment into which the control bacteria did not pass. Wolbach found the *Spironema* in the act of passing through the pores by preparing a thin section of the filter which had been employed for the filtration. He is of the opinion that the infectivity of a filtrate is due to the presence of the regular organism and not to that of filterable granules, as is assumed by others. C. Fraenkel failed to obtain an infective filtrate with any filtrates whatever.

*Spironema icterohemorrhagic* was found by Inada and his associates<sup>79</sup> to pass through the Berkefeld filters, grades V and N. Out of 28 experiments the filtrates were found to be infective for guinea pigs fifteen times. It is not stated whether the filtrate contained the *Spironema* in a regular form. Huebener and Reiter<sup>209</sup> also report the filterability of the virus of Weil's disease prevalent in Germany. Since they claim a spiral organism, *Spirochæta nodosa*, found by them to be the etiological agent, the same organism must be considered filterable. It passes through the Berkefeld filters V and N. As mentioned elsewhere, *Spironema nodosum* (*Spirochæta nodosa*) is probably the same organism as *Spironema icterohemorrhagic* (*Spirochæta icterohemorrhagic*) discovered a year earlier by Inada.

*Treponema pallidum* and *Treponema pertenue* are unable to pass through any bacteria-proof filters when filtered by the usual processes [application of a vacuum or a positive (compressed air) pressure]. Metchnikoff, Klingmüller and Baermann,<sup>210</sup> Casagrandi and de Luca,<sup>211</sup> and many others, established this fact in the case of syphilis, and Castellani in the case of yaws. On the other hand, the pallidum can grow through the pores of the Berkefeld filters,<sup>57</sup> grades V and N, and appear in the filtrate when provided with favorable cultural conditions for several days. On the fourth day the young forms commence to appear in the fluid which collects in the empty tube that is fitted up to receive the drops that fall by spontaneous diffusion without suction or pressure. This phenomenon, which was first noticed and utilized by myself when obtaining a pure culture from mixed cultures, has since been confirmed by Nakano<sup>212</sup> and others.

There are yet other spiral organisms which are of great interest from the standpoint of filterability. Thus, Wolbach and Binger described *Spirochæta elusa*<sup>213</sup> and *Spirochæta biflexa*,<sup>214</sup> which they obtained in a filtrate of stagnant water taken from the shores of a fresh water pond in the vicinity of Boston. The first was cultivated but the second was not. With the culture of *S. elusa*, which measures about 0.5  $\mu$  wide and 20  $\mu$  long with an average of six to eight curves, they were able to demonstrate the organism in the filtrate within about fifteen minutes. The filtration was made by suction with the Berkefeld filters, V and N. The organism is provided with one terminal flagellum at each end and is extremely motile. *Spirochæta biflexa* is a much more delicate organism. Another filterable organism, morphologically considered a "*Spirochæta*" in the loose sense of the term, was obtained by Wolbach and his asso-

ciate, from human feces. In explaining the filterability of these rather coarse spiral organisms, which are larger than many bacteria, Wolbach considers their plasticity to be one of the important factors.

#### CULTIVATION.

Only a comparatively limited number of "spirochætes" have been cultivated on artificial media. Of the free-living varieties *Spirochæta plicatilis* was cultivated by Zuelzer<sup>22</sup> in a flask containing  $\frac{3}{4}$  liter of stagnant lake water and  $\frac{1}{4}$  liter of water to which a certain amount of hydrogen sulphide had been added. According to this procedure the flask is hermetically (anaerobically) sealed after the inoculation, and hydrogen sulphide occasionally introduced. The role of  $H_2S$  is to produce sulphur by oxidation, ( $H_2S + O = H_2O + S$ ). By this means the organism can be kept in culture for an indefinite period. Wolbach's *Spirochæta elusa* was cultivated on a hay infusion (aerobically) where it propagates indefinitely. This organism is not allied to Ehrenberg's organism, but appears more like a *Spirillum*. No culture has been obtained of the molluscan *cristspira*. Of the *Spironema* group several varieties have been cultivated. Attempts at the cultivation of *Spironema novyi* by Norris, Pappenheimer, and Flournoy<sup>215</sup> were partially successful in that these investigators were able to notice a definite multiplication of the organism in human or rat citrate blood at room temperature within 24 hours, a second generation cultivated in a similar medium bringing about the same increase the next day. A third generation was not obtained. Occasional multiplication of the *Spironema* in a defibrinated blood had previously been shown by earlier investigators (Lachmann, Albrecht, Gerhardt). Cultivation of the blood *Spironema* in the strict sense of the term was achieved by the writer<sup>216, 217</sup> for the first time by employing a culture medium containing a piece of fresh tissue (rabbit's kidney, etc.), and ascitic fluid (10 to 12 cm. deep). This medium provides a condition that I proposed to designate as an *acrotropic anaerobiosis*; that is, a strictly anaerobic state is produced around the base of the fresh tissue, while the top of the ascitic fluid column has access to a certain quantity of oxygen. The whole medium may be covered with a layer of sterile paraffin oil in order to prevent evaporation of the fluid and this regulates at the same time the amount of oxygen admitted.

When the medium is inoculated with a minute quantity of the *Spironema*-containing blood and then incubated at  $37^\circ C$ . the organism multiplies steadily until every field will show numerous motile specimens which may occur singly, in pairs, or in chains of three or more individuals. The height of multiplication is reached within four or six days, and a sudden degeneration of the organisms sets in on the seventh to the ninth day. By making subcultures on the fourth or fifth day the culture can be carried on indefinitely. It was found that the success or failure greatly depends upon the suitability of the ascitic fluid samples. A sample which forms a loose fibrin web with the fresh tissue (rabbit's kidney) within 24 hours at  $37^\circ C$ . gives the best empirical results. The addition of glucose or peptone seems to hinder the growth of the *Spironema*, and sterilization by filtration or fractional heating also impairs the nutrient value of the medium. Invasion of the culture by any other bacteria quickly destroys the culture; in other

words, no mixed culture has been obtained. So far I have been able to cultivate *S. recurrentis*, *S. novyi*, *S. duttoni*, *S. kochi* and *S. gallinarum* by the same method. Plotz<sup>218</sup> successfully applied this method in order to obtain a culture directly from patients in Bulgaria suffering from the European relapsing fever. According to Hata,<sup>219</sup> instead of the fresh tissue and ascitic fluid, a medium consisting of a piece of blood coagulum and serum of the horse may be satisfactorily used for cultivating *S. recurrentis*. At the temperature of 35°C. the culture in this medium remains actively motile for a month (Hata). None of the spirochetes causing septicemia in man or birds have been cultivated on a solid medium, and nothing is known about such colonies. In a fluid medium they produce a diffuse opalescence, but no definite change of the medium is noticed. No odor or gas or change in the reaction has been detected in the culture. Their virulence remains unattenuated for many generations of artificial cultivation.

From the oral cavity *S. vincenti* and several spiral organisms have been obtained in pure cultures by various investigators. Tunncliff<sup>220</sup> considers *S. vincenti* and *Bacillus fusiformis* to be identical, but different phases of development and under varying conditions. All these organisms are strictly anaerobic and can be cultivated by the usual anaerobic methods on solid media (glucose agar with or without animal proteins). They form definite colonies comparable to any other bacteria and some are putrefactive or acid-producing organisms. I am inclined to regard them as allied to *Spirillum* rather than to *Spironema*.

*Spironema icterohemorrhagicæ* has been cultivated by Inada and Ito in the same medium as was originally employed by the writer for the cultivation of *S. recurrentis*, *S. duttoni*, *S. gallinarum*, etc. Ito<sup>221</sup> later succeeded in cultivating the organism on agar or gelatine containing human or guinea pig defibrinated blood in the ratio of equal parts or one to two of blood and agar or gelatine. The organism is said to grow readily on these media at a temperature of 26° to 37°C. A good growth takes place even at room temperature. Characteristic cultural features, such as gas or odor production, colonies, turbidity, etc., have not been recorded. In the fluid as well as in the solid media no visible growth was obtained. The culture is said to remain virulent for many generations and live over one month in a solid medium when kept at about 15° to 26°C.

In proportion to its clinical importance, *Treponema pallidum* has ever since its discovery in 1905 been the subject of correspondingly more numerous investigations. Volpino and Fontana<sup>222</sup> observed a temporary increase of the organisms after a piece of syphilitic tissue had been put in human serum or defibrinated blood and then incubated at 37° C.; but no culture was obtained. Lebailly<sup>223</sup> claims to have kept alive the pallida in the syphilitic fetal tissue for 15 days when put in human serum at 37°C. Levaditi and McIntosh<sup>224</sup> inoculated the inactivated human serum with the expressed serum of a syphilitic lesion of a monkey containing a few pallida, and, after sealing it in a collodion sac, introduced it into the peritoneal cavity of a monkey. It was taken out after a month and was found to contain numerous motile pallida along with certain contaminating bacteria. The contents of the sac cultivated *in vivo* could be successfully transferred from one sac to another for many passages with the same result. The impure pallida cultivated by this method were avirulent for monkeys. Mühlens

and Loche<sup>225</sup> failed to confirm the above findings. In 1909 Schereschewsky<sup>226, 227</sup> claimed to have succeeded in starting an impure culture of *Treponema pallidum* by implanting a semisolidified, clear horse serum with a piece of chancre or condyloma inserted several inches below the surface. The serum commenced to liquefy around the tissue and within several days more liquefaction took place. On examining the fluid or solid medium about the tissue he found very numerous actively motile spiral organisms resembling the pallidum. Some of them were coarser and less regularly curved and looked like *S. refringens*. An enormous number of cocci or bacilli were also present. The culture gave off an intensely offensive odor. Subcultures were carried on indefinitely. The impure culture was avirulent for experimental animals. About the same time Mühlens<sup>228</sup> obtained a pure culture of an organism from a syphilitic lymphadenitis of man by first using Schereschewsky's medium and then transferring the culture to another kind of solid medium consisting of horse serum and agar. In the latter he succeeded in purifying the *Treponema* from the contaminating bacteria. Notwithstanding the fact that the organism was derived from a material in which the pallidum would be the only small *Treponema* and in spite of its great resemblance to the pallidum, Mühlens' culture has certain characteristics which, as will be seen later, render the organisms distinguishable from the pallidum cultures which were obtained by others (Noguchi,<sup>229, 230</sup> Sowade,<sup>231</sup> Tomaszewski,<sup>232</sup> Bæslack,<sup>233</sup> Zinsser, Hopkins, and Gilbert.<sup>234</sup> Thus the organisms isolated by Mühlens were avirulent, produced a strong odor, and could grow from the beginning in a horse serum agar without the addition of any fresh tissue. W. H. Hoffmann<sup>235</sup> (1910-1911), a coworker of Mühlens, obtained several strains which were identical with that of Mühlens, except for the fact that he was able to produce in the rabbit's testicle a somewhat acute or subacute inflammation by injecting a large quantity of solid culture.<sup>236</sup> His description of the experiments leaves the syphilitic nature of the lesion indefinitely established. The extract of the organism acted as an antigen in the Wassermann reaction, as was also shown by Schereschewsky<sup>237</sup> in the case of his impure culture; but Mühlens as well as Schereschewsky obtained similar results when extracts of other bacteria were used. Recently Zinsser and Hopkins have confirmed the nonspecific nature of so-called antigens in this type of complement fixation. Bruckner and Galasesco<sup>238</sup> (1910) and Sowade<sup>231</sup> (1911) reported the successful inoculation of rabbits by means of their impure cultures (Schereschewsky medium) given intratesticularly and intracardially. Sowade claims to have produced generalized syphilis by the intracardial injection into half grown rabbits.

During 1910-1911 I was engaged in cultivating *T. pallidum*. Unlike the previous investigators, I had chosen the testicular syphiloma of rabbits as the material for cultivation for the reason that this material affords a constant and unlimited supply of a practically pure pallidum and as many strains simultaneously as one desires to try. Besides the rabbit strains being already acclimatized to the animal, this would more readily take when a culture derived from this source is to be tested for its virulence. After unsuccessful attempts to cultivate the pallidum in all the various media, previously reported suitable for cultivation of the pallidum, and a large number of culture media and conditions having also

failed, the following two methods were found to yield a positive growth of the organism on an artificial medium. As has been mentioned elsewhere, neither method is a perfect one and only a limited percentage of attempts is ever successful. The inconstant results are due partly to the different resistance offered by various strains to the artificial cultivation and partly to certain still unknown factors which enter into the composition of the media. At all events, the greatest difficulty in cultivating the pallidum is to obtain the first growth. As the number of generations increases the organism acquires an easier growth, and after a period of years of life in the culture, the organism becomes quite saprophytic and may grow even without the addition of fresh tissues. The strict requirements demanded by anaerobiosis and by the reactions and compositions of the media become more and more lax, until the culture may adapt itself to a great many cultural conditions. The two methods above mentioned are (1) a fluid medium consisting of a suitable sample of ascitic fluid or sheep serum water (Hiss) with the addition of a piece of freshly removed kidney or testicle from a normal rabbit; and (2) a solid medium consisting of a mixture of ascitic fluid and agar with the addition of a piece of fresh tissue as above described. The use of the fresh tissue seems to offer twofold advantages: first, as an oxygen absorbent as originally recommended by Th. Smith,<sup>239</sup> and secondly, as a source of nutrient substances needed for the pallidum. The first method (fluid medium) is applied exclusively for the cultivation of the testicular pallidum from rabbits, and the second (solid medium) is only used to cultivate the impure material derived directly from human syphilitic tissues. The first method requires an anaerobic apparatus, as a complete removal of oxygen from the atmosphere in which the cultivation is to be carried out is essential, while for the solid medium a layer of sterile liquid paraffin poured on the top of the culture medium suffices to prevent evaporation and possibly to minimize the diffusion of oxygen into the medium. The requisite anaerobiosis is produced by the fresh tissue which lies at the bottom of the tube. I shall not enter into any technical details, but suffice it to say that nearly a dozen strains were obtained within the last few years by the use of these two methods. The strains obtained by means of the fluid medium remained for many generations unadaptable to the solid medium to which they finally grew. On the other hand, the strains grown on a solid medium could readily be made to grow when suitable conditions were provided.\* Impure pallidum cultures in a fluid medium can be purified by allowing the pallidum to grow through the pores of a Berkefeld filter. Before the associating bacteria pass, the pallidum will appear in the filtrate (by gravitation) probably on the fourth or fifth day. Some of the strains of *T. pallidum* obtained by these methods were virulent to rabbits and monkeys when tested within a few months. The lesions produced were typical in every respect, although once the organism had entered the animal body it resisted recultivation just as much as before the first cultivation. In this respect they differ from the strains of W. H. Hoffmann,<sup>236</sup> who was able to cultivate the organisms back from the lesions into the horse serum agar without the addition of any tissue. His strains produced a strong offensive odor when recultivated. The strains cultivated in my laboratory did not and still do not give any

\*Fluid medium method.



offensive odor such as is described by Mühlens and W. H. Hoffmann. As has been stated no growth could be obtained without the aid of fresh tissue during the first year after these strains were isolated. Nor could they be induced to grow on a plain horse serum agar of Mühlens or semicoagulated horse serum of Schereschewsky. Since attention had been called to the differences which existed between the cultures of Mühlens and Hoffmann and my own cultures, later investigators gave special attention detecting any possible production of a peculiarly offensive odor. Sowade, Tomaszewski, Bæslack, Nakano, Zinsser, Hopkins, and Gilbert failed to find any such characteristic odor in their strains. Erich Hoffmann<sup>240</sup> considers that the cultures of Mühlens and W. H. Hoffmann either contained the pallidum and a second odor-producing organism, or were not *Treponema pallidum* at all, since there exist certain pallidum-like, easily cultivated saprophytic treponemata which in pure cultures produce a strongly offensive odor (*T. microdentium*, *T. mucosum*, *vide infra*).

That the cultivated strains of *Treponema pallidum* gradually become tolerant to various media and conditions has been strikingly demonstrated by the recent investigations of Zinsser, Hopkins, and Gilbert.<sup>234</sup> Thus the investigators found that a pallidum strain which they had isolated by the original fluid culture method, described by me, gave a good growth after the tenth generation in fluid media containing different kinds of autoclaved tissues of rabbits and a mixture of slightly acid meat infusion broth with heated sheep serum. This strain likewise grows well in symbiosis with *staphylococci*, *streptococci*, *Micrococcus candicans* and *Bacillus fecalis alkaligenes* added to the sheep serum agar mixture without tissue. Addition of dead staphylococci had the same effect as symbiosis. The gelatinized horse serum or sheep serum with or without the tissue proved to be a good medium for the growth of this strain. They have obtained the same results with two other strains which were isolated in my laboratory several years ago. The fact that during the first few years after isolation all my pallidum strains failed to grow on various media similar to those now successfully used by Zinsser, Hopkins, and Gilbert seems to indicate that rigid parasitic properties of the organism have gradually deteriorated due to the artificial cultural conditions, some undergoing the changes more abruptly than others. It is not at all improbable that in time the saprophytized strains of *Treponema pallidum* will adapt themselves to still simpler ordinary culture media. It is to be desired that efforts be directed toward improving the condition under which the organisms can be kept as nearly natural as those in living tissues, since the results obtained with completely denatured material might be quite different from those derived with the less modified material.

In summing up the pallidum cultivation one may say that the methods hitherto proposed are still imperfect and that much patience is still demanded in order to isolate a strain. Some strains remained persistently uncultivable in my hands. The strains isolated by Mühlens may have been *Treponema pallidum*; but there was no way of proving this, as his culture, which was avirulent, possessed certain properties inconsistent with those of the pallidum as subsequently defined by other investigators. The first instance, therefore, of a successful cultivation of *Treponema pallidum* was that which was carried out at the Rockefeller Institute in

1910-1911, in which were brought out not only the demonstration of the pathogenicity of the organism isolated, but also the studies of other biological characteristics of the culture.

*Treponema pertenue*<sup>241</sup> was also successfully cultivated in 1911 by the same method as that given for the cultivation of the pallidum. The material used for this work was in the form of a testicular lesion of experimental yaws in the rabbit and was supplied by Captain Nichols. The organism possessed the same cultural characteristics as the pallidum, but it was probably slightly thicker and less regularly curved. Preliminary attempts to produce lesions in rabbits ended negatively, and the strain was lost before further comparative studies could be undertaken.

Several spiral organisms were isolated from unclean lesions around the genital region. *Spironema refringens*<sup>242</sup> and *Treponema calligyrum*<sup>104</sup> (from a condyloma) were cultivated by me in the pure state by methods similar to those used for the pallidum and pertenue. Levaditi and Stanesco<sup>243</sup> obtained impure cultures of *S. gracilis* and *S. balanitidis* by means of gelatinized horse serum. A spiral organism, *S. phagedenis*, was also obtained by me in a pure culture from a phagedenic ulcer on the genitalia of a woman, but its systematic affinity is quite uncertain.<sup>95</sup> *T. calligyrum* is slightly coarser than the pallidum, but is apt to be mistaken for the latter in cultures. It grows easily in tissue-free media.

From dental deposits of normal oral cavity *Treponema macrodentium* and *T. microdentium*<sup>102</sup> were cultivated in the pure state by means of the same methods, and *Treponema mucosum*<sup>103</sup> from the scraping of the pyorrheal gum. The microdentium and the mucosum appear very similar but can be distinguished by the production of a thin but tenacious mucin in the culture of the latter. When the culture gets old, both of these give a strong, somewhat offensive odor. This faculty to decompose the proteins (thus causing a slight turbidity in the fluid media) makes this culture readily distinguishable from the pallidum cultures, because the latter do not produce such an odor. Morphologically, they bear a great resemblance to the pallidum, although their curves are set somewhat more closely than in the pallidum. The macrodentium is more difficult to cultivate and, according to my experience, requires fresh tissue in the culture media. Morphologically, it is coarser than the pallidum and its serpentine movements and irregular, stretchable and wider curves are characteristic enough to distinguish this species from other varieties. It does not produce an odor.

Mühlens and Hartmann<sup>244</sup> succeeded in 1906 in obtaining a pure culture of *S. dentium* in the horse serum agar medium of Mühlens. In their culture they recognized a minute form of Koch's *S. dentium* type and another which approached the dimension of Hoffmann-Prowazek's *S. media* type. They suggested the possibility of these representing different stages of development or even a sexual differentiation. It appears as though their so-called pure culture may have contained more than one species. No admixture of tiny and coarse forms has been observed in the culture of the macrodentium or microdentium. The dentium culture of Mühlens produced a strong offensive odor.

#### IMMUNITY AND IMMUNIZATION.

The very name, relapsing fever, suggests the possibility of the development of some sort of protective power in the infected hosts against a third attack. In

fact, the second attack is often milder than the first and a third relapse is rare. Persons who have had the fever are usually immune to subsequent infection for a period of several years. The same is true of the African tick fever although repeated recurrences are more frequent in this instance. Susceptible animals, such as monkeys, mice, and white rats, enjoy a period of immunity extending over about three months after recovering from the second attack. In rats no relapse has been observed. In the fowl and geese, similar immunity follows recovery from spironematosis. The studies of various investigators, especially Gabritschewsky, Pfeiffer, Novy and Knapp, Manteufel, Marchoux and Salimbeni, Levaditi and Manouélian, Prowazek, Neufeld, and others, have contributed in explaining the mechanism upon which the immunity depends. Gabritschewsky<sup>245</sup> demonstrated the presence of a specific antibody against *S. recurrentis* in the blood of convalescent patients by mixing it with the spironema-containing blood *in vitro*. The destruction of the organism occurred within a short time when the mixture was kept at a temperature of 37°C. This author considered that the development of a germicidal substance in a patient's blood was the cause of the crisis and subsequent immunity. He also recognized the appearance of a similar specific immune substance in the geese recovering from the attack of *S. anserina*. The convalescent *recurrentis* blood had no effect upon the organisms of goose fever and the *anserina* blood did not affect the organisms of the relapsing fever. The phenomena observed were agglutination, immobilization and dissolution of the organisms when mixed with their correspondingly specific bloods. Gabritschewsky produced an immune serum by injecting the horse with a spironema-containing blood. It was tested by Löwenthal in 83 cases, and in 39 cases (47%) no relapses occurred, while in 140 untreated cases, 65 had three attacks (46.5%). Novy and Knapp<sup>15</sup> confirmed and greatly extended the experimental part of Gabritschewsky's work and pointed out that the protection afforded by active as well as passive immunity is not wholly dependent upon the germicidal property, but also upon the immune bodies, since a comparatively weak germicidal blood may protect the animal against the infection in small quantities. Besides, Novy and Knapp hold the role of the phagocytes (mononuclear, but not polynuclear) to be very important, as they ingest the dead as well as the enfeebled spironemata under the influence of immune bodies. Levaditi and Manouélian<sup>246</sup> suggest the existence of an opsonin in this phenomenon. Manteufel<sup>247</sup> believes the lysis of the spironemata in the immune serum to be due to the cooperation of complement and a specific amoebocyte. The rapidity and intensity with which the spironemata are destroyed within the peritoneal cavity of actively or passively immunized rats is variable. In the peritoneal cavity of a hyperimmunized animal the organisms become granular in from two to five minutes, while in rats recently recovered from the attacks, the organisms are ingested in fifteen minutes. In passively immunized rats the spironemata are first agglomerated and temporarily immobilized and this is followed by the appearance of some leucocytes on the scene; but the effects of the immune substances gradually wear off within about an hour. The leucocytes disappear in 30 minutes (Novy and Knapp). The germicidal and bacteriolytic actions are parallel. The duration of passive immunity in rats is less than forty days while that of the active immunity lasts nearly four months. Novy and

Knapp succeeded in preparing in rats by means of hyperimmunization a powerful immune serum which contained in each cubic centimeter 500 immunity units; that is, 0.002 c.c. of the serum was able to protect the rat against 0.1 c.c. of the infective blood showing 10 to 50 spironemata per field (2 mm. objective). In ordinary recovered rats there were only about 2 immunity units per cubic centimeter. The use of the immune blood from a hyperimmunized rat prevented the infection in the rat and cured it on its onset, but a greater amount is found necessary in order to obtain similar results in monkeys and mice as these animals are subject to a relapse after the treatment. Novy and Knapp suggested the inoculation of the *Spironema* during the apyretic period in order to increase the amount of immune principles in the victim's system and thereby ward off a relapse. They found an interesting phenomenon; i. e., the injection of too much immune blood proved to be less effective than a moderate quantity. This was explained by assuming the production of a specific precipitin which acted as an anticomplement. In regard to the use of hyperimmunized blood serum in human relapsing fever, they calculated that about 375 c.c. of a serum such as mentioned in the experimental part would be necessary and that the future of a serotherapy much depended upon the success attained in cultivating the organism in an artificial medium in large quantities. As a matter of fact we have been able to collect large quantities of comparatively pure organisms from each of the cultures of *S. recurrentis*, *S. duttoni*, *S. novyi*, *S. gallinarum*, etc., for various purposes (immunization, vaccinothrapy, etc.). In the serum of those who had just recovered from the relapsing fever, a complement fixation principle was demonstrated by Kolle and Schatilloff,<sup>248</sup> and Korschun and Leibfried.<sup>249</sup> The reaction was said to be positive after the second attack.

In Weil's disease Inada and his coworkers found the presence of a specific spironemalysin in the serum of convalescent man or guinea pig. The immune bodies develop after the second week of the disease and may be still present in individuals who had the attack more than four years previously. The Pfeiffer phenomenon is easily demonstrated by using the organ (liver or kidney) emulsions rich in the spironemata or a culture and the immune serum in the peritoneal cavity of the guinea pig. These investigators immunized goats and horses with the cultures of the causative agent (*S. icterohemorrhagiae*) for a period of more than a year and succeeded in producing a serum which prevents the infection against the lethal dose in guinea pig in the amount of about 0.001 c.c. The clinical experience of this serotherapy which has now extended over many hundreds of cases proves to be highly encouraging.\*

The question of immunity in syphilis is rather imperfectly understood. In human subjects it was once assumed that after the first infection, complete immunity occurred as evidenced by the extreme rarity of a reinfection. Later investigators seem to consider this assumption as incorrect inasmuch as it was based upon the fact that the syphilitic individuals do not a second time contract a chancre or show a general skin eruption in spite of exposure to such an infection. This fact does not, however, necessarily denote immunity in the usual sense of the word. This state of refraction to the second infection is said to be due to the pre-existence of the same virus in the same individual who no

\*Personal communication, soon to appear in print.

longer reacts to the second inoculation with the original intensity or vigor, and the condition is designated by Neisser as "Anergie." At the same time Hutchinson showed the possibility in rare instances of an autoinoculation, while Finger and Landsteiner<sup>250, 251, 252</sup> believe that a superinfection may take place in certain syphilitics. The effect of a superinfection may be a purely local manifestation or it may be subsequently followed by generalization; or it may again cause a general mobilization of the virus without a local manifestation. The character of the lesions produced by superinfection agrees with that of the lesions peculiar to different stages of the disease. If it occurs during the secondary stage, the superinfected lesion will be a papule or other exudative product, and if during the tertiary stage, the result will be a gummatous product. This alteration of various tissues of a syphilitic individual in their reactivity to the syphilitic virus is designated as "Unstimmung" by Neisser who regards this condition as a morbid state of the tissues brought about by the pressure of *Treponema pallidum*. There once prevailed a vague impression that when cutaneous tissues are extensively involved there is less likelihood of the visceral organs being invaded by the syphilitic virus and vice versa,<sup>253, 254</sup> but there is no experimental proof to support this contention. Since the introduction of salvarsan and its derivatives in the treatment of syphilis, the instances of reinfection with typical or sometimes atypical chancres are not so rare, thus indicating that after a cure has been effected the human body reacts in the usual or nearly usual manner.<sup>255</sup> This also points to the absence in such cases of any lasting immunity after the first infection has been eradicated. A thorough investigation is required in order to ascertain whether or not a certain degree of immunity develops in some of the cured cases, thereby affording protection. In some ways the question of immunity in syphilis is comparable to that in protozoan diseases, in which, though latent, no typical infection can be reinduced until the first attack is completely cured, and where no congenital immunity has yet been demonstrated.

Let me now review the situation of the immunity question in experimental syphilis. Metchnikoff and Roux, Neisser and Bruck, and others found that monkeys that have once been infected with *Treponema pallidum* may prove refractory to subsequent inoculation. Metchnikoff<sup>256</sup> thought he succeeded in protecting a monkey against the infection by inoculating it with an attenuated living virus which was no longer able itself to produce typical reactions. That the vaccination against syphilis was not equivalent to that against variola in its fundamental principle was later demonstrated by Neisser and others, who were able to show that the monkeys that had been "vaccinated" with an attenuated virus and which were rendered "immune" to the subsequent inoculation with a fully virulent material were harboring the infection in various localities escaping the usual clinical detections. Thus the emulsion prepared from the bone marrow, spleen, etc., of the "vaccinated" animals was able to infect new susceptible animals. This phenomenon is similar to the state of anergy observed in syphilitic human subjects. Fontana,<sup>257</sup> Uhlenhuth and Weidanz,<sup>258</sup> Bertarelli,<sup>259</sup> Truffi,<sup>260, 261</sup> and others, pointed out that a rabbit which carries syphilitic keratitis in one eye is not refractory or immune to the infection in the other eye. A rabbit, one of whose testicles is infected with *T. pallidum*, offers no greater

resistance in the other which may be infected with the virus at any stage of orchitis preceding that on the opposite side. Tomaszewski<sup>262</sup> thought that a skin infection produced in rabbits in which scrotal lesions had been persisting for about two months was much milder than in normal animals. According to personal observations, a rabbit in which a syphilitic orchitis, or keratitis, or scrotal chancre has been cured either spontaneously or through the administration of salvarsan enjoys no perceptible immunity to syphilis. Truffi repeatedly inoculated rabbits with a fetal liver emulsion containing an abundance of *T. pallidum*, but found no immunity to develop. Uhlenhuth and Mulzer immunized rabbits with the testicular pallidum emulsion without obtaining any decisive result, although in some cases they thought it exerted a beneficial influence upon the syphilitic process. In my personal experience it has been found that the susceptibility of the rabbit to syphilis is decidedly diminished in some animals by immunizing them with *T. pallidum* for several months. With a strain which gave 100 per cent takes in normal rabbits' testicles, only about 60 per cent positive results were obtained in the immunized animals. This tends to show that the lower percentage of positive takes in the immunized rabbits may be due to the destructive influence of the treatment upon the invading pallida. But it was also found that in the immunized rabbits in which the inoculation succeeded, the symptoms were not any milder. In fact, not only were the local reactions just as marked as in the control animals, but there was a tendency to the formation of generalized lesions. In two of the rabbits scrotal lesions developed after the intravenous inoculation of a virulent strain. It appears that an incomplete immunization exerts an adverse influence on the defensive factors of the rabbit. This phenomenon finds verification in the work of Grouven and Sowade,<sup>263, 264</sup> who recommended for the animal a few preliminary intravenous inoculations of the pallidum in order to insure a generalized infection through a subsequent intracardial introduction of the organisms in huge quantity. I also endeavored to ascertain whether a local administration of devitalized pallida (killed at 60° C.) on many successive occasions will not bring about a state of local immunity to *Treponema pallidum*, but my results were rather unsatisfactory, for the reason that the testicular parenchyma which was repeatedly inoculated with the pallidum emulsion underwent gradual atrophy, and the resulting hard fibrous structure was no longer a suitable test object for this fastidious parasite. Nevertheless I was able to produce small nodular lesions in two out of several rabbits so treated. Moreover, reinfection of the same tissues (cornea, testis, and skin) after a spontaneous or chemotherapeutic healing has been found possible as long as the suitable structures of the tissues are preserved.

Our knowledge pertaining to the immunity phenomena *in vitro* is of more recent date, for the test tube experiments with *T. pallidum* were made possible since the discovery of the organism and were particularly favored by the successful cultivation of the parasites on artificial media. Attempts to demonstrate the presence of a specific agglutinin for *T. pallidum* in the sera of human and experimental syphilis were made by Hoffmann and Prowazek,<sup>265</sup> Herxheimer and Löser,<sup>266</sup> Hoffmann,<sup>267</sup> Brönnum and Ellermann,<sup>268</sup> Babes and Panea,<sup>269</sup> Metchnikoff and Roux, Landsteiner and Mucha,<sup>270</sup> Zabolotny and Maslakowetz,<sup>271</sup>

and others with the pallida derived from the syphilitic tissues. Their experiments were indecisive, owing to the difficulty found in obtaining a pure material free from various tissue constituents. Uhlenhuth and Mulzer<sup>189</sup> found no agglutinins to be formed in the sera of the rabbit, goat and monkey after repeated intravenous injections of the rabbit's testicular emulsion rich in the pallidum. In 1910-1911, soon after obtaining pure cultures of *T. pallidum*, we started the immunization of rabbits with different strains of the organism. In the sera obtained from the immunized rabbits we were able to demonstrate the presence of the specific agglutinins and complement-binding principles for the cultivated pallidum strains. We were unable to produce with the sera any unmistakable agglutination of the pallidum derived directly from the syphilitic orchitis of the rabbit, but I considered this to be due to the simultaneous presence of tissue debris and other cellular elements which may have interfered with the agglutination phenomenon. These sera were not strictly specific, but contained a small quantity of agglutinins for other treponemata obtained in pure cultures. There were also a sufficient number of specific complement-binding bodies, but there was at the same time a more or less definite group reaction for other treponemata. The work was continued later by Akatsu at my laboratory with similar results. He was able to obtain a serum which could agglutinate the pallida in a dilution of 1:50,000.

In order to know whether syphilitic human sera have any definite agglutinating and complement-binding properties, a number of sera obtained from various stages of syphilis were examined with pure cultures as well as with the tissue pallidum derived from rabbit testicles. All the experiments were unsatisfactory, owing to the difficulty experienced in reading the reaction in the case of agglutination and also owing to the high anticomplementary powers of the antigens and the feebleness of the reaction in the case of the complement fixation test, except in the case of the pure culture antigens which fixed complement with the immune rabbit as well as with some of the syphilitic human sera (chiefly late and tertiary cases). According to our experiments, there is a certain degree of group reaction for the other treponemata (*T. calligyrum*, *T. microdentium*, *T. mucosum*, and *S. refringens*).

Kolmer<sup>272</sup> first described the agglutination of a pure culture of *Treponema pallidum* by the sera of rabbits injected with a living and heat-killed culture furnished by our laboratory. His results show that normal rabbit sera do not agglutinate the culture pallidum in dilutions as low as 1:20, while the sera of immunized animals produced agglutination in dilutions as high as 1:1,280. No definite agglutination was observed with human syphilitic sera in a dilution of 1:20 or higher. Nakano<sup>273</sup> also reported the presence of agglutinins in the sera of rabbits injected intravenously with a pure culture in dilutions from 1:10 to 1:70. Kissmeyer<sup>274</sup> immunized rabbits with a pure culture of *T. pallidum* and was able to obtain agglutinations in dilutions as high as 1:200,000 to 1:500,000 of the immune sera, while the sera from individuals with primary, secondary, tertiary and congenital syphilis contained agglutinins for the pallidum in dilutions of 1:100 and higher in a percentage of from 40 to 60 out of 59 cases. Normal human sera may agglutinate the pallidum in dilutions as high as 1:50. Zinsser and Hop-

kins<sup>275</sup> state that normal rabbit serum may agglutinate the pallidum in dilutions lower than 1:10, but the sera of their immunized rabbits (intravenous injections of the pallidum cultures) agglutinated it in dilutions as high as 1:2,000. They added that the normal as well as certain syphilitic human sera may agglutinate the culture pallidum in emulsions. Zinsser, Hopkins, and McBurney<sup>276</sup> failed to observe any agglutination when the pallida from human lesions were mixed with the immune sera (rabbits and sheep) produced with the culture pallida. Zinsser and Hopkins<sup>277</sup> demonstrated the treponemicidal bodies for *T. pallidum* (cultivated) in the immune serum produced by them.

In the sera of animals experimentally infected with syphilis the presence of specific complement-binding antibodies for *T. pallidum* has not been satisfactorily proved. It is true that we were able to demonstrate the positive complement fixation in the sera of animals immunized with cultivated treponemata, but this does not hold good when dealing with the syphilitic animal sera and the virulent pallidum strains found in tissues. On the other hand, these syphilitic sera do bind complement when mixed with pure cultures, not only of *T. pallidum*, but also of various bacteria, such as colon bacilli (Zinsser and Hopkins). Undoubtedly the phenomenon is nonspecific but pathognomonic as is the Wassermann reaction which is caused by certain lipoidal substances. These cultures must serve as the containers of the similar lipoids. Indeed, Craig and Nichols<sup>278</sup> long ago showed that the alcoholic extracts of the pure pallidum and pertenuae cultures produced almost equally strong complement fixation when mixed with the human syphilitic sera, giving a positive Wassermann reaction with pure lipoidal antigens derived from other tissues. In a word, a syphilitic animal may give a positive complement fixation with various lipoids without at the same time containing any specific antibody for *T. pallidum*. In human syphilitic sera the same is also true, except in the sera of certain late and tertiary cases in which there may be a positive reaction due to the specific antigens and antibodies in the strict sense of Bordet-Gengou's phenomenon.<sup>279</sup>

The nature of the Wassermann reaction in the sera of human and experimental syphilitic subjects is still unexplained, but one fact has been established; viz., that it is due to a peculiar change of the sera not specific for syphilis; it occurs in yaws, leprosy, trypanosomiasis, malaria (febrile period), and sometimes in malignant tumors. The fact that so many lipoidal substances as well as certain salts (sodium taurocholate, sodium cholate, etc.) derived from different sources can bring about a positive fixation precludes any strict specific antigen-antibody reaction. According to personal observations the lipotropic complement fixation reaction is not present in immune rabbit sera which have been obtained by injecting the pallida repeatedly, and which contain a large number of specific complement fixation bodies from the pallidum strains employed for their production.

Closely related to immunity is the question of allergy in syphilis. From the chronic nature of the disease many investigators considered the possibility of its occurrence at one stage or another. Jadassohn, Meirowsky, Ciuffe, Fontana, Neisser and Bruck, and others made numerous observations which rendered the presence of allergy still more probable. These investigators were



handicapped by not having a pure culture of *T. pallidum*. Soon after the isolation of the pallidum strains, study of this subject was made in human syphilis with the pure material. In the meanwhile it was ascertained experimentally that the prolonged treatment of rabbits with intravenous injections of the pure pallidum culture as well as with the organisms obtained direct from the rabbit orchitis lead to the production of a state of hypersensitiveness of the skin to the inoculation of the extract of a pure, heat-killed pallidum culture.<sup>280</sup> The reaction was found to be apparently specific for *T. pallidum*. There was no injurious effect following the injection into the rabbits of the heat-killed pallidum emulsion. The emulsion, since known as luetin, was employed as a means of diagnosing human syphilitic cases, with the result that the luetin reaction was found to be most frequently present in the latent, tertiary, and congenital syphilis cases where one would naturally expect most constantly to find the allergetic or hypersensitive state of the skin. As an auxiliary or supplementary factor in producing a positive luetin reaction, I have already pointed out that the pathological state of the skin of chronic syphilitic patients designated by Neisser as "Umstimmung" played a role in nearly 10 per cent of tertiary cases in which the skin reacted intensely to the inoculation of the control emulsion without the pallida. No efforts were made to explain this peculiarity of hypersensitiveness of the skin of certain syphilitics. But recent work of Camp<sup>281</sup> points out that the administration for many days of potassium iodide to a nonsyphilitic individual produces in the skin a hypersensitiveness to any trauma, including the inoculation of the luetin. Probably this finding may furnish the solution of the problem of Neisser's "Umstimmung," or at least of one of the contributing factors. The clinical evidence thus far accumulated seems to show, however, that in a large number of cases the luetin reaction was positive in spite of the fact that no iodide had been given during the period when the test was applied. Recently Akatsu,<sup>282</sup> at my laboratory, carried out several series of experiments regarding the influence of potassium iodide upon the reactivity of the skin of rabbits to the intradermal inoculation of the luetin, control fluid, and plain bouillon. The iodide was administered intravenously for a period of from 7 to 9 days, given in increasing doses of 0.5 to 2 c.c. of a 10 per cent aqueous solution. At the end of seven days or later the skin was tested for the luetin, control, and plain bouillon. It was found that the skin of normal rabbits did not react to the injections after the iodide treatment. There was no change in its reaction to the trauma. The skin of the rabbits which had been previously rendered hypersensitive to the luetin by means of prolonged immunization with pure pallidum cultures mostly remained the same, that is, it reacted to the luetin with the same intensity as it did before the administration of potassium iodide. Only in a few instances was the reaction somewhat intensified. There was no definite reaction to the control emulsion of plain bouillon. In some rabbits, in which the testicular orchitis after several months had shrunk to a small fibrous nodule, the luetin reaction was mildly positive, but the intensity of the reaction was but little influenced after the injection of potassium iodide, except in a few rabbits where the second tests came out more distinctly. The above findings show that the potassium iodide has no noticeable influence upon the reactivity of the skin of normal as well as of syphilitic rabbits. It would be interesting to

study whether in other spiro-nematoses (relapsing fever, tick fever, rat-bite disease, and infectious jaundice) there appears any skin allergy comparable to that described for other bacterial infections (typhoid, gonorrhea, etc.). In cases of yaws, the skin reacts to the intradermal inoculations of the luetin and of the framboesin with equal intensity and cannot be differentiated by this method (Baermann and Heinemann).<sup>283</sup>

The last and probably the most important field for medicine is chemotherapy. The inauguration of modern chemotherapy by Ehrlich is as interesting as it is romantic. It can be traced back to Schaudinn's suggestive but unsupported theory that the spirochætes represent a stage of the life cycle of trypanosomes, or at least were closely related to the latter. The introduction of organic compounds of arsenic into the treatment of trypanosomiasis was promising much when Schaudinn discovered *T. pallidum*, which he regarded as a protozoon allied to the trypanosomes. Ehrlich took up experimental chemotherapy in connection not only with the latter, but also with the newly discovered spirilloses, as he called them, including syphilis and the fowl fever caused by *S. gallinarum*. The achievement of Ehrlich and his collaborator Hata, in discovering salvarsan for the treatment of these two diseases, marks a new era in modern chemotherapy. To review this phase of the spirochæte problem would be out of the scope of my present paper. Suffice it to say that to the great pioneers, Schaudinn, Ehrlich, Metchnikoff, and Neisser, we owe an inestimable debt, not merely for their own researches, but also for re-kindling in us the sublime stimuli which have already inspired so many investigators to discover new facts, and which will continue to urge us still more to take up this task and to extend our knowledge regarding the classification, morphology, biology, pathogenesis, and experimental as well as clinical aspects of the microorganisms known as spirochætes.

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# THE EFFECT OF EXTENSIVE VENESECTION AND TRANSFUSION ON KIDNEY LESIONS IN SEVERE ACUTE MERCURIC CHLORIDE POISONING\*

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THE manner in which the destructive changes found in kidney, colon, and liver are induced in mercuric chloride poisoning is at this date still a subject of controversy. Whatever the mechanism, it is true that death in man, in a large majority of cases, results from the destructive action, direct or indirect, on renal parenchyma. McNally and I<sup>1</sup> have shown that irreparable kidney parenchyma degenerations (as far as the life of individual renal cells is concerned) can be produced within 3 minutes after the administration per os of a massive toxic dose of mercuric chloride. Chemically the mercury can be detected and is present in the circulating blood in amounts that can be determined quantitatively within a short period of time. Renal cells, like other tissue cells, die and are replaced many times in conditions of normal health or in disease. Even in sublimate poisoning, heroic attempts at extensive regeneration and substitution of the destroyed renal cells are made by the kidney as has been shown by Heineke<sup>2</sup> and many others. When, however, the destruction of the renal epithelium involves tubules practically en masse, and when the remaining undestroyed excretory cells have undergone such severe degeneration that they are unable to functionate, the individual is usually doomed to death from the resulting uremia before new cells capable of functioning can regenerate, or before the injured cells can rehabilitate themselves sufficiently to again take up their own and the added work of their destroyed neighbors.

Modern therapy in the treatment of acute mercurial poisoning has concerned itself principally with attempts at the removal of the unabsorbed poison from the gastrointestinal tract. Lieb and Goodwin<sup>3</sup> have shown that mercury, after its absorption, is excreted into the stomach only to be reabsorbed unless removed by aspiration. Flexner and Sweet<sup>4</sup> have shown that mercury absorbed from the gastrointestinal tract is in part excreted by the liver into the bile which conveys it to the small intestine where it is again absorbed and taken back to the liver. Thus the brunt of the final elimination is borne by such organs as the kidney and colon. It is because of this repeated reabsorption by some organs that the complete elimination of the mercury is such a long extended process. Clinicians have profited by past experimental work, and at present, frequently repeated gastric lavage and colonic flushing or continuous irrigation are yielding most gratifying results as evidenced by the work of Lambert and Patterson<sup>5</sup> and many others. Despite the fact that the lives of some individuals are saved in this way, the prognosis in many cases must still be considered dubious. The fact that, within a few moments after the ingestion of a massive toxic dose of mercuric chloride, sufficient mercury has been taken into the circulating blood to produce most extensive kidney degeneration would indicate that, in conjunction with our present

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therapeutic measures, an additional therapeutic method which would aid in the removal of the mercury already absorbed and present in the blood would be of inestimable value. Its value would be proportionately enhanced if this removal could be accomplished speedily and through some other portal than an excretory organ. As has been indicated the vicious circle established by the continued reabsorption following elimination by other organs increases and prolongs the task set for the kidney. Rosenbloom,<sup>6</sup> in extensive separate analyses made of the organs and body of a girl suicide who died ten days after taking 15 grams of mercuric chloride, found that there was still present in the entire body 175.39 mg. of mercury. Almost  $1/3$  of this amount, 52 mg., was contained in the blood alone. Almkvist<sup>7</sup> has found experimentally the greater bulk of mercury contained in the animal body to be present in the blood, lymph, and "tissue juices." If then one could remove from the blood all of the absorbed mercury in addition to preventing further absorption of the mercury at its portal of entrance (most frequently the stomach), the kidney and other vital organs would be spared the further onslaught of the toxic agent contained in this blood and they would recuperate more rapidly. That this thought has occurred to many workers is evidenced by a large volume of experimentation recounted in the earlier literature. In most instances it was attempted to render the mercury contained in the blood inert by changing it to an insoluble compound. Various chemicals have been injected intravenously or administered by mouth for this purpose. Results have been variously reported. Therapeutically all of these methods have been abandoned by most clinicians who have attempted them. If, as it would seem, it is impossible to remove mercury satisfactorily in this way, one other method, to date apparently untried, still remains open to us. Recent successes and simplifications in the methods of transfusion would seem to indicate the way to this last avenue of approach—complete substitution of the mercury-containing blood by nonmercury-containing blood. Alternating copious venesection followed by transfusion of normal blood would assuredly aid in removing the toxic substance. Whether the degree to which the kidney could be spared would warrant such a procedure seems on the surface problematical, even doubtful. Viewed in its most favorable light, little could be hoped for unless one were certain that there would be no further absorption of mercury to contaminate the newly transfused blood. At best, then, this procedure could be only a supplementary therapeutic aid. Incidental factors such as the initial damage done to the kidney and the completeness and rapidity of the removal of the circulating mercury must assuredly be of much moment in a problem of this type. With an hypothesis of this character, admittedly of dubious prospect, yet not without its ray of promise, the following experiments were undertaken:

A series of 14 dogs and 24 rabbits were used. One-half of this number were used as controls. For each animal transfused a separate control of approximately the same size, weight, and sex, was chosen. Where there was a difference in weight, the larger animal was chosen as the control. Young dogs weighing from 4 to 7 kilos were used. In our previous work McNally and I<sup>1</sup> found that mercury is absorbed and taken into the blood very rapidly when administered by mouth to an empty stomach, and that practically simultaneously tissue changes in remote organs appear. It was therefore decided to admin

ister the mercuric chloride to these animals subcutaneously. This assured absorption as rapid as that from an empty stomach. In addition, complete anesthesia to prevent loss of mercuric chloride by vomiting could be eliminated. Mercuric chloride in a slightly acid water solution (15 to 20 c.c.) was administered in amounts varying from 0.25 to 0.5 grams. This was injected subcutaneously in amounts from 2 to 5 c.c. in various parts of the body thus promoting most rapid absorption. The injections in the controls were in the same amounts and in corresponding subcutaneous locations. Blood corpuscles which had been previously obtained from normal dogs' blood, had been washed free from their plasma and had been kept in a solution of  $2\frac{1}{2}$  per cent dextrose in Ringer's solution at  $+4^{\circ}\text{C.}$  were used in the transfusion. The blood treated in this way was obtained from 1 to 3 days before it was transfused. The animal was transfused from 2 to 3 hours after the administration of the mercuric chloride. No preliminary examination was made to determine the presence of isoagglutinins or isohemolysins on the part of donor or recipient. The animal was transfused under ether anesthesia. Bleeding and transfusion was done by means of a 100 c.c. Luer syringe and glass cannula, via the right jugular vein. From 100 c.c. to 200 c.c. of blood were withdrawn at one time and followed immediately by slow indirect transfusion of an equal volume of the washed corpuscle suspension. This was continued until 400 to 700 c.c. of blood had been removed. In the smaller dogs the ultimate volume removed in this manner was equal to  $1\frac{1}{2}$  times the total normal blood volume for the animal. In the larger dogs this represented usually slightly less than the total amount of blood. From  $1\frac{1}{2}$  to 2 hours were usually occupied in making this blood substitution. The vein was tied and the skin was closed. The control animal received no treatment. Death invariably occurred within 18 to 24 hours. With one exception all of the transfused animals died 2 to 8 hours earlier than the controls. In the former instance the death of the control occurred 8 hours prior to that of the transfused animal. In the rabbit series blood from normal rabbits was obtained and washed as was the dogs' blood. Rabbits weighing from 1500 gm. to 2500 gm. and equally heavy control animals were used. Mercuric chloride in amounts of 0.01 gm. to 0.06 gm. was injected subcutaneously similar to the manner in which it was administered to the dogs. Because of the difficulty experienced in obtaining generous amounts of blood by venesection, the rabbits were bled by cardiac puncture. They were then transfused through a marginal ear vein with a suspension of washed corpuscles from an equal volume of normal rabbits' blood. In five rabbits and their five controls death occurred within 2 to 4 days. Three of the transfused animals lived from 10 to 20 hours longer than their controls; the remaining 2 were found dead in their cages 4 to 8 hours before the death of their controls occurred. Three rabbits died within a few hours following cardiac puncture and transfusion. At necropsy death was found to be due to a heart tamponade secondary to intrapericardial hemorrhage from the cardiac puncture wound.

A comparative study of the kidneys of these animals and their controls which seemed to warrant a continuance of the work will be discussed later. Had the mortality rate in these experiments been less discouraging there would still have been a most serious drawback to the practical application of this method of therapy, i. e., the difficulty experienced in obtaining donors for such large quan-

tities of blood. I therefore decided to attempt a removal *in vitro* of the mercury from the blood obtained by venesection, and to utilize this blood in a subsequent transfusion. In medico-legal investigations the presence of mercury is frequently determined quantitatively by electrolytic methods. A modification of the Wolff-Schneider<sup>9</sup> apparatus for this purpose was kindly designed for me by W. D. McNally. This consisted, as will be noted from the accompanying illustration, of a glass container for the blood. In this were immersed a funnel-shaped gold, and a spiral-shaped platinum electrode. Two dry cells in series, or a 4 volt storage battery were used. The platinum electrode was connected to the cathode, and the gold to the anode. In a series of preliminary experiments, blood from dogs poisoned with mercuric chloride was washed by the method previously cited. Fifty to 70 c.c. of such washed corpuscles when tested for mercury always gave a most marked positive Reinsch test. An equal quantity

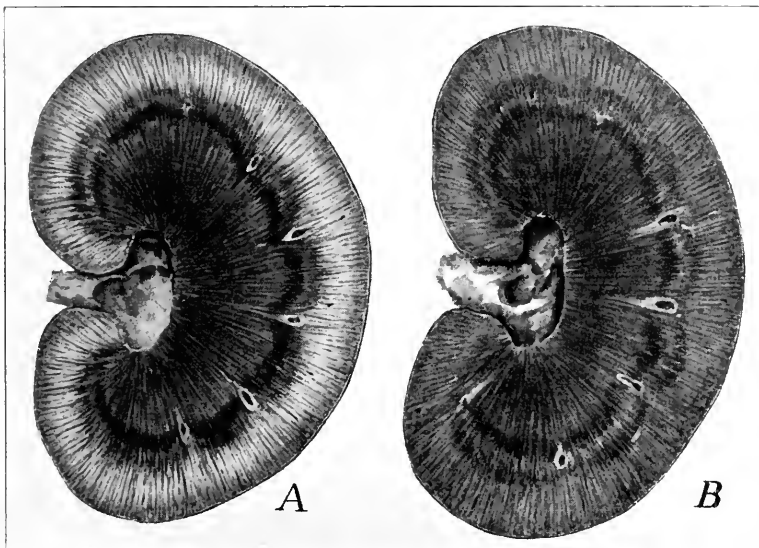


Fig. 1.—*A*. Kidney of control dog which received no treatment. Grossly the changes of fatty degeneration located in the epithelium of the ascending limbs of the loops of Henle are very evident. *B*. Kidney of dog treated by venesection and transfusion. This dog was approximately of the same age, of equal weight, and the same sex as the dog from which *A* was taken. He received a dose of mercuric chloride subcutaneously equal to that of *A*.

of such blood placed in this apparatus was negative for mercury by the Reinsch test after a current had been passed through it for 45 minutes. In careful hands it has been found that the Reinsch test will yield positive results where as little as 0.00001 gm. of mercuric chloride is present in 100 c.c. of suspected material. During this mercury-removal process bubbles of chlorine and other gases were liberated and rose to the upper surface carrying along some clumps of erythrocytes enmeshed in a small amount of fibrin-like material. After 45 minutes the contents of the reservoir were drawn off and filtered under sterile precautions through 8 to 10 layers of surgical gauze. When examined microscopically, the erythrocytes appeared decidedly crenated. There were no blood shadows, no fragments of corpuscles, and the corpuscles were not clumped. It was found, however, that from 30% to 50% of the corpuscles were lost during these manipu-

lations. Because of this the corpuscles were again washed and recentrifugalized. By withdrawing a sufficient quantity of the upper clear fluid after centrifugalization, the total volume of the corpuscle suspension was reduced until counts yielding from 7,000,000 to 8,000,000 erythrocytes per c.mm. were obtained. Three rabbits poisoned with mercuric chloride were exsanguinated and their blood treated in this way. Three normal rabbits weighing from 1400 gm. to 1500 gm. were then bled by cardiac puncture and 50 c.c. to 60 c.c. of blood was removed from each. These rabbits were immediately transfused through a marginal ear vein with an equal volume of the corpuscle suspension obtained from the blood of the poisoned rabbits. There were no untoward results. The rabbits, in collapse following the cardiac puncture, were on their feet within half an hour and during the following 20 days were apparently normal. They were subsequently used in other work. To each of three rabbits weighing 1500 gm. to 1600 gm. was then administered 0.01 gm. of mercuric chloride hypodermatically. After one hour they were bled by cardiac puncture to the extent of 50 c.c. of blood and immediately transfused through a marginal ear vein with an equal quantity of blood from normal rabbits. The blood removed by cardiac puncture from each rabbit was washed and freed of its mercury by the method described; the volume was adjusted to an 8,000,000 per c.mm. erythrocyte count. Enough normal rabbits' blood was added to each portion to bring the volume to 40 c.c. One to two hours following the first cardiac puncture the animals were again and similarly bled to the extent of 40 c.c. of blood and immediately transfused as before, this time, each with its own blood. This was the blood which had been obtained at the previous bleeding and freed of its mercury in the interim. Death in all three instances ensued within the next hour. Apparently the shock attendant upon two cardiac punctures was too great. The three control animals died within two to three days.

At necropsy in the rabbit series no striking differences were noted grossly in a comparison of the kidneys of the control and of those of the transfused animals. In the dog kidneys, however, relatively marked differences were apparent. The light ray-like streaks of fatty degeneration (microscopically usually limited to the ascending limbs of the loop of Henle) so characteristic of acute mercuric chloride poisoning in the dog, were in all instances less numerous and decidedly fainter in the transfused animals than in their controls. This was equally striking

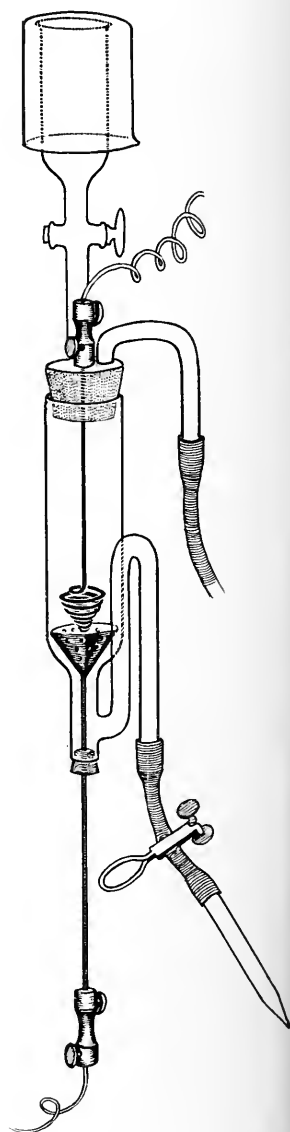


Fig. 2.—Apparatus for removing mercury electrolytically from the blood.

microscopically in Sudan III stained sections. In most instances fewer ascending limb tubules—numerically  $\frac{1}{3}$  to  $\frac{1}{2}$ —were involved, and the degree of fatty degeneration of the epithelium was far less advanced. Frequently only a few cells in the entire tubule presented these changes, whereas in the control animal every cell in an entire tubule was found involved, and this was practically uniform for all tubules of this type. No material differences, however, were found in the degree of degenerative change in the convoluted tubule epithelium. In both transfused and control animals severe parenchymatous degenerations were usually present in these tubules. The minute black globules and granules described by Almkvist<sup>7</sup> and others which are to be seen in parenchyma cells and blood and lymph channels in the kidney of mercuric chloride poisoned animals, were quite as abundant in the kidney of the transfused as in the nontreated animals.

#### CONCLUSIONS.

1. Copious venesection followed by transfusion of normal blood inhibits both qualitatively and quantitatively the characteristic degeneration usually found in the epithelium of the ascending loop of Henle in the kidneys of dogs acutely poisoned by mercuric chloride.

2. In desperate and other cases of acute mercurial poisoning, venesection followed by transfusion should be practiced in addition to other therapeutic measures now in use.

3. Erythrocytes, from the blood of rabbits, when suspended in Ringer's solution are apparently not injured by a 3 to 4 volt electrical current passed through the solution for  $\frac{1}{2}$  to  $\frac{3}{4}$  hour periods.

4. In this way metallic mercury can be removed in vitro from the blood of animals poisoned by mercuric chloride and this blood qualitatively freed from its corrosive contamination can again be utilized in subsequent transfusions.

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## RECENT ADVANCES IN THE STUDY OF AUTOLYSIS\*

BY MAX MORSE, PH.D., CHICAGO.

THE purpose of this brief communication is to present some of the more recent work concerning the behavior of intracellular enzymes, which is of more definite interest to the clinician. An exhaustive account of modern investigations in this field would involve lines of work, which, while of the greatest importance in themselves, are of lesser importance to the medical man. As an example, we may mention yeast autolysis, where the problem of hydrolysis and synthesis is being considered. Meager reports have been made concerning the role of yeast proteolytic enzymes in synthesizing proteins for human food; but interesting as these matters are, we shall confine the following pages to a more circumscribed field. For general considerations, the reader is referred to the excellent summary of Wells,<sup>1</sup> to the book of Carl Oppenheimer,<sup>2</sup> and to Vernon's article.<sup>3</sup>

It is widely accepted as a matter of demonstrated fact that autolysis; that is, the process of functioning of intercellular enzymes, plays a major role in pathology. Thus we find the statement made that "all absorption of dead or injured tissues seems to be accomplished by means of digestion by the enzymes of the cells and tissue fluids."<sup>1</sup> The removal of infarcted tissues, digestion of necrotic areas, involution of the uterus, liquefaction of nervous tissue, dissolution of pus, disappearance of pneumonic exudates, atrophy of the liver, and many other pathological phenomena are assigned to autolysis in causal relations.

Upon what basis do we rest our belief that intracellular enzymes are responsible for these processes? The evidence is *a posteriori* in each case. Ligate an organ from its blood supply and estimate the amino-acid content of the organ; amino-nitrogen has markedly increased.<sup>4</sup> Study newly formed pus and compare it with that which has existed for some time; amino-acid increase again in the latter case.<sup>5</sup> Excise an organ, say, the spleen. Grind it up, suspend it in water, and cover with chloroform and toluene, permit to stand a few days in a temperature of 37° C; dissolution and an increase, again, of amino-acids.<sup>6</sup> The evidence, then, is that inasmuch as there is an appearance of the end products of erepsin-like digestion, erepsin-like digestion has taken place.

However willing we may be to accept this analysis as final, we must answer the following questions before we may legitimately relegate the processes enumerated above to autolysis, as the term is defined:

1. Why is it that there is a wide discrepancy in rate between *in vitro* autolysis of brain, muscle, etc., and the instances referred to autolysis involving these tissues, mentioned above? The liquefaction of brain tissue is remarkable for its rapidity, yet brain tissue is the slowest in rate to autolyze<sup>4</sup> out of the body. The same statements may be applied to muscle, except that the discrepancy is less marked.

\*From the Nelson Morris Memorial Institute for Medical Research of the Michael Reese Hospital. Presented at the January, 1917, meeting of the Clinical Society of the Hospital.

2. Why is it that an actual acidity ( $\text{pH} < 7.07$ ) is necessary for *in vitro* autolysis,<sup>7, 8, 9</sup> while in no instance has a true acid reaction been attained in the organism save in the stomach and in the urinary bladder?<sup>10</sup> Certainly we cannot align uterine involution with autolysis, for the blood supply is intact and any tendency toward  $\text{pH} < 7.07$  is met by buffer salts, as Henderson<sup>10</sup> has shown. We may say the same of certain types of carcinomata, of hemorrhagic infarcts where total occlusion of the blood has not occurred, etc.

Until these discrepancies are explained, we must apply the Scots' verdict of not proved.

Modern studies of autolysis have approached more and more closely to the factors fundamental to the process. Up until the present time, for instance, it has been believed that the effect of acid, long known to accelerate *in vitro* autolysis, was directly upon the enzymes, activating them as we believe pepsin is activated by hydrochloric acid,<sup>11</sup> and as other enzymes are affected by reaction of medium. Bradley,<sup>12</sup> however, has shown that the effect of adding acids or "acid" salts concerns not only the *rate* but *the point of equilibrium of the reaction*; that is, not only is the rate accelerated, but the total quantity of end products of hydrolysis is greater than in the control. Now, Ostwald has demonstrated that a true catalyzer does not affect the point of equilibrium, but the rate alone, and for this reason Bradley has been led to examine the matter further. He has found that the addition of foreign proteins to an autolyzing digest gives the same picture as a digest where acid has been added. He likewise finds that when acid is added to such a digest, the rate and the point of equilibrium are not affected, meaning, as he believes, that no enzymic effect is obtained. Other reasons are given in the original papers, to which the reader is referred. The matter is important because there is no essential difference between the natural accumulation of acids in dying tissues (hydroxy acids, ketone acids, etc.) and the addition of acid from without, artificially. Bradley has attempted to formulate an explanation of starvation metabolism, acidosis, and other pathological states in terms of intracellular enzymes. To this theory the writer has objected as far as interpretation is concerned.<sup>13, 14</sup>

Another theory concerning autolysis is that which has been erected to explain the acceleration at the inception of autolysis where oxygen supply is eliminated. W. E. and E. L. Birge,<sup>15</sup> on studying the effects of oxygen upon pepsin and trypsin, found that the action of these enzymes was suppressed in an atmosphere of oxygen gas and purely as a matter of analogy, they assumed that autolyzing enzymes reacted similarly. That there is no basis for such an assumption has been shown by the writer,<sup>16</sup> who studied the effects of oxygen gas, oxides and peroxides upon autolysis. If oxygen or other oxygen-bearing reagents affect intracellular enzymes in any way, it is an acceleration rather than a retardation, so that any theory based upon the belief that oxygen, when present, as in normal tissues, inhibited the action of these enzymes, while, on interfering with the blood supply, oxygen supply is interrupted and the inhibitory mechanism removed, is untenable. From the time of Hoppe Seyler and his pupil Lubavin,<sup>17</sup> who first recognized autolysis as a ferment action, this erroneous theory of oxygen relation has been held.

What really happens when the blood supply is cut off from an organ is the accumulation of  $\text{H}_2\text{CO}_3$  and perhaps of acids of incomplete combustion, produced intramolecularly, such as hydroxy acids (lactic, etc.), ketone acids, etc., so that autolysis proceeds in an acid medium.

Regarding the factors operating to permit autolysis in some tissues at a much greater rate than in others, the writer has eliminated two. In the first place, inasmuch as the number of nuclei per cubic millimeter in gland tissue is much greater than the number in tissues such as muscle, nervous, etc., which autolyze more slowly, it is a tempting hypothesis that this factor of nuclear components may be a potent one. To solve at least one portion of the problem, the writer<sup>16</sup> added nucleic acid salts, such as sodium nucleate to autolyzing tissue and determined that there is no acceleration in the presence of the nuclear compounds. There remains, of course the possibility that the enzyme component of the tissues autolyzing at faster rate is greater than that of the more slowly digesting material and the writer is at present attempting to determine this point.

Again, it is possible that some of the tissues which undergo autolysis at greater rate produce more acid in a given time than those autolyzing more slowly. The writer has studied this in a preliminary way, but to an extent sufficient to warrant the statement that as far as brain compared with liver is concerned there is no basis for the assumption. Using the Sørensen colorimetric method with Hynson-Wescott-Dunning tubes with phenolsulphonphthalein as indicator, the tissues were dialyzed within 5 seconds after their excision from the rabbit which had been killed by an occipital blow, against 0.8% NaCl, pH=7.0. The results in both instances ran parallel and approximated, essentially, the findings reported before.<sup>7</sup> At the end of an hour, the reaction gave approximately the same in both cases, pH = 6.4. Inasmuch as the rate of autolysis during this time is a function of the concentration of hydrogen-ions to at least a considerable extent, it seems unlikely that acid development affects the quantitative results in a differential way among the tissues.

It must be admitted, then, that much remains to be accomplished in the study of the fundamental factors in autolysis before we are at liberty to assign limitations of the participations of this process in pathology.

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# PERNICIOUS VOMITING WITHOUT ACIDOSIS ASSOCIATED WITH A DISTURBANCE OF THE AMMONIA-UREA QUOTIENT OF THE BLOOD\*

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SEVERAL forms of persistent vomiting, not connected with any definite demonstrable pathological lesions have been described at different times. Many of these attacks have been attributed to a condition of acidosis, as for example, the cyclic vomiting of children. Others have been explained by pregnancy, pelvic disorders in women, and still others, the so-called idiopathic vomiting of adults, for example, remain unexplained.

It has been the privilege of one of us, during the past few years to have observed two cases of pernicious vomiting in adults, not associated with any demonstrable lesions of the body which could account for the condition, nor with any acidosis so far as was determined. Since these patients presented practically identical clinical pictures, and since one of them came to autopsy, while the other was made the subject of a limited metabolic study by means of some of the newer methods recently placed at the disposal of the clinician, we feel that these cases, taken together, may be worthy of report.

The symptoms of both of these patients were practically identical, consisting in each case, primarily, of severe and persistent periodic vomiting associated with a certain degree of mental unrest, irritability, and towards the termination of the disease, of stupor. Delirium was not noted. No fever, chills, jaundice, nor abdominal tenderness or rigidity was noted in either case. Death terminated the disease in one case, while the other recovered after a serious and prolonged illness.

The cases in detail are as follows:

F. S., 39 years of age, single, a seamstress by trade. Entered the hospital complaining of vomiting which had come on suddenly without apparent cause. Family history negative. Past history negative, except for the continuous use of alcohol in the form of both whiskey and beer for about ten years. The present illness had come on three days before admission, apparently without any exciting cause.

Physical examination on admission revealed nothing of note. No abdominal tenderness or rigidity was noted. Liver apparently not enlarged. Patient was rather dull and stupid mentally, but became irritable and querulous when aroused. Gastric analysis showed an absence of free HCl with an average total acidity of 10. No other abnormalities. Duodenal contents and stools repeatedly negative. Blood showed a slight relative anemia (red blood cells, 4,000,000) and a white count of 9,000 with a normal differential count. Wassermann negative. Urine showed a specific gravity of from 1015 to 1030. Reaction faintly acid to litmus. No albumin, casts, or acetone bodies upon repeated examina-

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tions. Death occurred two weeks after admission, the patient gradually becoming weaker, and the vomiting continuing at intervals throughout the course of the disease. Death was apparently caused by gradual cardiac failure. No delirium noted at any time.

Autopsy showed absolutely nothing to account for death, a rather marked degree of cloudy swelling of the liver being the only gross abnormality noted. Microscopic sections of brain, kidneys, pancreas, and liver failed to add any information as to the cause of death.

The second case, seen only recently, who has apparently recovered, is that of a man, white, 60 years of age, a railroad conductor by occupation. Entered the hospital complaining of persistent vomiting. Family history negative. Past history negative except that like the preceding case, he has been a steady drinker for a long period of years, averaging according to his statement, from four to five drinks of whiskey per day for forty years. The present illness began one week before admission with an attack of faintness and dizziness which was so severe as to cause him to fall to the floor (but not to lose consciousness). Subsequent to this attack, periodic vomiting made its appearance and has persisted ever since, coming on at irregular intervals. According to the patient, he has to lie on his right side constantly in order to prevent vomiting.

Physical examination shows a fairly well nourished individual. Heart and lungs negative; peripheral arteries sclerosed; liver apparently not enlarged. No abdominal tenderness or rigidity. Systolic pressure 150; diastolic 105. Mentally, the patient was rather irritable and querulous, although his reasoning power seemed to be fairly normal. Pulse rapid on admission, averaging 110. No fever noted at any time. *Gastric contents* showed an absence of free hydrochloric acid on several occasions with an average total acidity of eighteen. No other abnormalities. Duodenal contents and stools negative. Numerous *urine* examinations showed a specific gravity of from 1010 to 1025, no casts, albumin, or acetone bodies at any time. Twenty-four hour output averaged from 1,000 to 1,200 c.c. Phenolphthalein excretion 55 per cent in two hours. *Blood examination* showed a total red count of 4,700,000 and a white count of 10,000. Differential normal. Wassermann negative. Examination of the *spinal fluid* revealed no abnormality.

The patient remained in practically the same condition for about two weeks, vomiting at frequent intervals throughout this time. Vomiting gradually became less severe until the attacks ceased entirely. Nausea without vomiting then ensued for several weeks but gradually disappeared. The patient is still rather weak and emaciated, but is now able to eat freely of almost any food and is bright and cheerful mentally.

In view of the fact that a case had been observed previously, which was very similar to this one clinically, and that no lesions had been found at autopsy which could account for the symptoms, although the disease was so severe as to cause death, we determined to investigate the metabolism of this case as thoroughly as possible with the methods at our command. Inasmuch as Williams<sup>1</sup> has shown that in the pernicious vomiting of pregnancy a definite increase of the ammonia percentage in the urine is noted, as compared with the total nitro-

gen output, we were particularly interested in the urea-ammonia quotients of the blood and urine in this case.

The following tests for blood and urine urea were carried out by means of the Van Slyke<sup>2</sup> method. Ammonia was tested for by means of the Folin aeration method. The CO<sub>2</sub> content of the alveolar air was determined by means of the Fridericia method.<sup>3, 4</sup>

The following results were obtained:

Blood:	Urea	36 mg. per 100 c.c.	(October 26)
	"	34 " " " "	(November 3)
	"	39 " " " "	(November 22)
	"	36 " " " "	(December 3)
	"	35 " " " "	(December 14)
	Ammonia	23 mg. per 100 c.c.	(October 26)
	"	17 " " " "	(November 3)
	"	12 " " " "	(November 22)
	"	8 " " " "	(December 3)
	"	0 " " " "	(December 14)
Urine:	Urea	13.6 gm. output in 24 hours	(October 28)
	"	13.2 " " " "	(November 3)
	"	12.8 " " " "	(December 3)
	Ammonia	0.8 gm. in 24 hours	(October 28)
	"	0.5 " " " "	(November 3)
	"	0.38 " " " "	(December 3)
Alveolar Air:		October 26, 45 mm. of Hg.	
(CO <sub>2</sub> Tension)		November 3, 43 mm. of Hg.	
		November 26, 46 mm. of Hg.	

An analysis of the above examinations reveals a very marked increase in the ammonia percentage of the blood. While, according to Folin and Denis<sup>5</sup> as well as all others who have worked upon this subject, only slight traces should be found in the blood normally—such slight traces, in fact, as to be scarcely perceptible by means of the ordinary quantitative test; in this case almost as much of ammonia as of urea was present in the blood. The urea percentage of the blood was found, however, to be only slightly above the limits of normal. The urine showed a slightly diminished urea output with a slightly increased ammonia excretion.\* Examination of the CO<sub>2</sub> content of the alveolar air revealed absolutely no suggestion of acidosis, nor did the examination of the urine for acetone bodies reveal any sign of their increase at any time. Apparently, therefore, acidosis could be eliminated as a cause, *per se*, of the blood ammonia. Since it is in the liver that much, if not all, of the ammonia caused by protein metabolism is believed to be changed into the nontoxic urea,<sup>6, 7, 8</sup> the question suggests itself to us as to the possibility of a functional derangement of that organ being a factor in accounting for the symptoms and metabolic abnormalities found in this case.

Feeling that it would be desirable to apply the same methods which we had used in studying this case, to other individuals, both normal and abnormal, we have carried out similar studies routinely upon twenty different individuals. An analysis of this list reveals several pathological conditions associated with

\*While these findings according to Ambard's law indicated a slight deficiency of the kidneys, this deficiency was certainly not sufficient for the symptoms noted in this case.

an increase of blood ammonia; this increase being found particularly in individuals suffering from failure of cardiac compensation. Acidosis has, however, been present at the same time in all of these cases. None of our normal cases (seven) have shown any demonstrable quantities of ammonia in the blood by the Folin method.

While we do not feel, from this limited study, that any sweeping conclusions can be drawn as to the origin and significance of an increased ammonia content of the blood, we feel that at least three facts have been demonstrated:

First: Increased amounts of ammonia appear in the blood in certain pathological conditions independent of any condition of acidosis, and are presumably due to functional disturbances of the liver.

Second: In the estimation of blood urea as a diagnostic procedure, simultaneous estimations of the blood ammonia should be carried out, since, otherwise, a considerable error in the urea readings may be produced in certain pathological conditions.

TABLE I

ALGER CO.

DISEASE	UREA (GM.) PER 100 C.C. OF BLOOD	AMMONIA (GM.) PER 100 C.C. OF BLOOD	TOTAL OUTPUT OF UREA IN URINE IN 24 HOURS IN GM.	TOTAL OUTPUT OF AMMONIA IN URINE IN 24 HOURS IN GM.	CO <sub>2</sub> TENSION OF ALVEOLAR AIR	ACETONE BODIES IN URINE	REMARKS
1 Normal.....	0.02	None	.....	.....	46	.....	.....
2 Normal.....	0.027	None	.....	.....	42	.....	.....
3 Normal.....	0.018	None	.....	.....	40	.....	.....
4 Normal.....	0.02	None	.....	.....	39	.....	.....
5 Normal.....	0.038	None	.....	.....	41	.....	Arteriosclerosis.
6 Normal.....	0.022	None	.....	.....	40	.....	.....
7 Normal.....	0.014	None	.....	.....	43	.....	.....
8 Nephritis.....	0.068	0.003	7.2	0.6	26	None	Dyspnea.
9 Nephritis.....	0.095	None	4.8	0.3	40	None.....	No dyspnea.
10 Uremia.....	0.12	0.006	2.3	1.3	20	Acetone and diacetic.....	Cheyne-Stokes respiration.
11 Nephritis.....	0.068	None	3.	0.5	22	None.....	Dyspnea marked.
12 Myocarditis Nephritis	0.13	None	4.	0.8	29	None.....	Dyspnea marked.
13 Nephritis with Uremia.	0.131	None	.....	.....	27	None.....	Moderate dyspnea
14 Myocarditis?.....	0.031	0.017	.....	.....	28	Acetone and diacetic.....	Pulsating Liver
15 Myocarditis?.....	0.021	0.0015	8.5	1.3	22	Acetone.....	Dyspnea.
16 Myocarditis?.....	0.014	0.006	3.3	0.5	32	None.....	Chronic Passive Congestion of Liver
17 { Alcoholic Cirrhosis of liver.....	0.036	0.01	12.2	0.49	40	None.....	No dyspnea.
17 { Carcinoma head of pancreas. Marked jaundice.....							
18 { Catarrhal jaundice.....	0.012	None	.....	.....	46	.....	No dyspnea.
18 { Syphilis of liver.....							
19 Catarrhal jaundice.....	0.015	None	.....	.....	39	.....	No dyspnea.
20 Syphilis of liver.....	0.024	None	.....	.....	43	.....	No dyspnea.

Third: A case is reported characterized by nausea, vomiting and mental abnormalities (irritability, depression) in whose blood a large amount of ammonia was constantly found in spite of an absence of acidosis. This phenomenon we believe to have been due to a functional insufficiency of the liver.

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## LABORATORY METHODS

### THE USE OF FEHLING'S SOLUTION IN THE DETERMINATION OF BLOOD SUGAR\*

BY HUGH MCGUIGAN, PH.D., M.D., CHICAGO.

UP to the present time no agent has been so much used in blood sugar determinations as Fehling's solution. If one examines the Fehling solutions made up by the various formulas recommended, a great variation in the alkalinity is found. For example, the United States Pharmacopeia VII used 125 grams of KOH in 500 c.c.; United States Pharmacopeia VIII reduced this to 75 grams; and the present United States Pharmacopeia IX reduces this to 50 grams. This makes the alkalinity of the mixed fluid vary from 12.5% to 5% KOH. The U. S. Department of Agriculture Bureau of Chemistry, 1910, No. 107, revised, used 125 grams to 500 c.c.; Mathews' Physiological Chemistry uses the same strength; Fehling's original solution, final mixture was about 5.6% which he afterwards reduced to about 4.75% NaOH. Claude Bernard first used about 11.5% and later about 6% NaOH of the mixed fluids. Many other examples might be cited. On the whole, however, the final mixed fluid in common use varies from 5% to 12.5% KOH, and these limits may be extended.

If one examines the percentage of sugar reported in the literature purporting to be the normal level of the blood sugar, it will be found ranging all the way from about .03% or .05% to 0.150% for dogs.

In recent work in which was used both Benedict's method and the Bertrand method the author and his coworkers were struck by the differences in the yield. When for instance the Bertrand method gave .02% the Benedict method gave 0.1%. The explanation for this difference was found, and it is believed, also for the variations in the normal sugar concentration of the blood reported in the literature.

Our recent method of preparing the blood for the Bertrand titration was to remove the protein with picric acid. The picric does not interfere with Fehling's solution, and need not be removed until the precipitated cuprous oxid is filtered off, when the picric can be washed out readily. In precipitating the blood we used 10 c.c. of blood, laked, and made this to 150 c.c. with saturated picric solution. This was then filtered and an aliquot part used. We used 100 c.c. of the filtrate = 6.66 c.c. of blood.

The Fehling solution most used was the Allihn modification of Fehling solution, except that NaOH was used for KOH, and the solution was diluted with an equal quantity of water, as used by Morris.†

\*From the Department of Pharmacology, Northwestern University Medical School.

†Morris: Clinical Laboratory Methods, p. 39.

The following is the exact formula :

I.	Copper sulphate	34.65 gm.
	Water to	1000. c.c.
II.	Rochelle salt	173.0 gm.
	Sodium hydrate	125.0 gm.
	Water to	1000. c.c.

When these are mixed, the NaOH concentration is 6.25% which is approximately that recommended by Hammersten, Remsen, and others. Sixty c.c. of this mixed fluid and 100 c.c. of the picric filtrate were heated to boiling and then the Fehling poured into the picric solution, and the mixture kept boiling for two minutes. With these volumes there is no bumping, and the changes due to evaporation are reduced to a negligible quantity. This solution used on known dextrose solutions in water will give good results so it is considered satisfactory in blood sugar work.

In seeking for the reason of the great difference between this method and the Benedict colorimetric method, we naturally suspected a sugar of the maltose type and so hydrolysed, as we thought, and obtained theoretical results. There were many facts, however, especially the short time necessary to complete the "hydrolysis" which made us doubt this explanation, and we finally found that the addition of the acid directly to the Fehling solution, i.e., lessened alkalinity, gave the same result. This striking fact was fully confirmed by the use of a Fehling solution in which the alkali strength approximates that given in the present United States Pharmacopeia which is the approximate strength used by most investigators who have reported the higher levels of the normal blood sugar.

There is a striking difference between a blood filtrate and a water solution of sugar. In a water solution of 0.1% dextrose we have never found more than 10% to 15% variation no matter what the alkali concentration of the Fehling solution used. In blood filtrates, however, the strong alkalies in Fehling prevent the precipitation of the copper.

Similarly dextrose added by *glycolysed* blood or to yeast fermented sugar filtrates cannot be recovered completely when strongly alkaline Fehling's solutions are used, but can be recovered completely when the final strength is about the equivalent of 5% KOH, as recommended in the present Pharmacopeia. Increasing the copper has much the same influence as decreasing the alkalinity. It is apparent, therefore, that the relation of the copper to the alkali is important. It is usually only the alkali, however, that is changed.

An example of the differences due to the variations in the alkalinity of Fehling's solution will suffice: A dog was bled from the jugular, no anaesthesia, the blood was oxalated and precipitated as above:

I.	1. Lewis-Benedict method gave	0.106%
	2. Fehling 6.25% NaOH in the mixed solution gave	0.018%
	3. Fehling 2.5% NaOH in the mixed solution gave	0.090%
	4. Fehling 2.0% NaOH in the mixed solution gave	0.105%

The dilutions here were made by adding Rochelle salt solution to Solution II above. In this way the alkali only varied.

## II. Dog bled in same way:

In this solution the copper is about double that in I.

1. KOH in Fehling's	10% $\text{CuSO}_4$	7%	0.09%
2. KOH " "	5% " "	7%	0.157%

## III. In water solutions, the copper concentration remaining the same:

5% KOH = 0.115

12.5% KOH = .106

or a variation of relatively slight importance.

This variation in the alkalinity of Fehling's solution seems to be the main cause of the great differences in the literature regarding the level of the normal blood sugar. The error is due to the assumption made by all writers; viz., that the sugar in a protein-free blood filtrate will act toward Fehling's solution as a solution of dextrose in water. Such an assumption is apparently untenable. Why a weaker alkaline Fehling should give a greater yield than a strong one is hard to explain. It seems to be because such a solution is more sensitive. It is not because the stronger alkali destroys the sugar, for two reasons: first, in a water solution of dextrose the difference between the stronger and weaker alkaline Fehling is relatively slight; second, if blood filtrate and stronger Fehling be boiled for two minutes and then the alkalinity be reduced by the addition of acetic acids, the yield of the weaker alkaline Fehling can be obtained. These results obtain no matter what agent is used to remove the proteins. Alcohol, sodium sulphate, colloidal iron, and picric acid all give a higher blood sugar yield when a 5% KOH Fehling is used than when 12.5% KOH is used. The weaker solution gives results which agree more closely with Benedict's colorimetric method, and is, I think, much more accurate than that obtained from the more strongly alkaline fluid.

## THE PRESERVATION OF ERYTHROCYTES OF A KNOWN GROUP FOR ISOAGGLUTININ GROUP DETERMINATION\*

By M. G. WOHL, OMAHA, NEBR.

THE most efficient method for testing donors and recipients for blood transfusion is that of Moss as modified by Minot. A complete and accurate account of the technic employed has been given recently by Brem.

I have found this procedure very simple and practicable. However, a great difficulty that one encounters is the necessity of having on hand serum and red blood corpuscles of a known group, two or three. The serum, if kept sterile, will retain its agglutinative power for a month or longer. The suspension of red blood corpuscles, however, will not keep longer than forty-eight hours. The preservation of the red blood corpuscles then is a desideratum of great importance. With this aim in view the various methods devised for preservation of sheep cells for the Wassermann reaction have been applied to citrated suspension of human red blood corpuscles of known groups, two and three. The ad-

\*From the Pathological Department, Nicholas Senn Hospital.



dition of formaldehyde to sodium citrate solution has given us very satisfactory results. The formalized cells preserved as long as four weeks showed neither hemolysis nor lost the property of becoming agglutinated with the serum of the proper group. The serum of the known group was preserved in sterile Wright's blood capsules.

The stock solution is made up by adding 0.5 c.c. of 40 per cent formaldehyde solution to 500 c.c. of 0.85 per cent salt solution containing 2 per cent sodium citrate. To 1 c.c. of this solution is added three drops of blood and the suspension is preserved in cotton stoppered test tubes. When needed, the corpuscles are brought into suspension by gently shaking the test tube.

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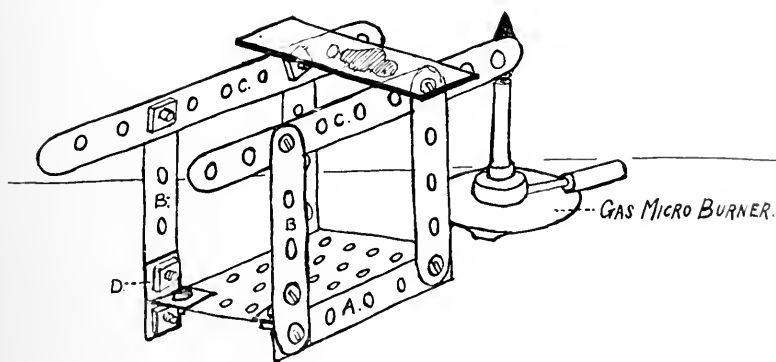
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## CONSTRUCTION OF APPARATUS USING THE STRUCTURAL STEEL TOY

BY SIMON B. KLEINER, M.D., NEW HAVEN, CONN.

THE piece of apparatus illustrated is for holding slides while they are being stained for microscopic examination. It is of practically the same design as those found in many pathological and clinical laboratories, but it has been constructed from the most inexpensive outfit of miniature structural steel, the entire cost of the apparatus being only ten cents.



The mode of construction of the apparatus is self-evident. It consists essentially of a base (A) with four uprights (B) which support two parallel bars (C). Small elbows (D) hold two of the uprights rigid. If desired, the uprights may be bent slightly to approximate the parallel bars to within a space of about one and a quarter inches of each other.

The slides to be stained are placed upon the parallel bars and can then be stained and heated. The apparatus is small enough to be placed in a pan to catch the stain and washings.

The author does not claim any originality for this apparatus, but wishes rather to suggest the use of the structural steel toy to construct laboratory apparatus, surgical splints, and orthopedic appliances. This toy can be purchased in any city, at a very moderate price, and working models of apparatus, or apparatus itself, of neat design, can be easily constructed with it.

Moreover, any design found unpractical can be taken down quickly, and an entirely different model can be as readily built again.

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## CLARIFICATION OF COMPLEMENT IN RELATION TO ITS PRESERVATION\*

BY NORMAN E. WILLIAMSON, M.D., STOCKTON, CALIF.

THE usual directions for the preparation of complement include the pipetting of the clear (?) serum after its separation. Such a serum will of course contain millions of cells. It is well known that human serum may contain amboceptors for the guinea pig cells. Guinea pig complement would not be particularly active against guinea pig cells. Nevertheless, in order to prevent the possibility of a false positive from such an action, I am in the habit of centrifugating my complement to perfect clearness, that is from twenty minutes to half an hour.

I find that complement so clarified will preserve its properties with slight gradual loss for days in the ice box without freezing. It will lose about one-third of its strength in five days. I arbitrarily discard complement which is weaker than two-thirds of the strength of average complement, that is 0.02 c.c. for a unit in the Noguchi system. Thus, if the material proves to be stronger than average on titration, it is still useful for many days. Titration at time of use is, of course, necessary.

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\*From the State Hospital, Stockton, Calif.

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## EDITORIALS

### *Resolutions Passed by the Faculty of the University of Michigan Medical School, April 2, 1917*

THE Faculty of the University of Michigan Medical School assembled on the above mentioned date and passed the following resolutions:

1. It is the opinion of the Faculty of the University of Michigan Medical School that in meeting the demands for medical officers in the National service, the military authorities should give first preference for enlistment to the members of the medical classes of the past two years, viz.: 1915 and 1916.

*Note.*—These young men have recently finished their medical courses and having taken in part or altogether their hospital training, should have the latest and best information in scientific medicine, and not having as yet established themselves in practice, are best fitted to be selected for military service.

2. In view of the probably urgent demands for trained medical men, the Faculty of the University of Michigan Medical School desires to place itself on record as being ready and willing to make its courses of instruction continuous through the summers of 1917 and 1918. This proposition will be submitted to the various State Boards of Licensure for their approval.

*Note.*—If this provision goes into effect, a week after the close of the present session, the session of 1917-18 will begin. Those who are now Juniors will become Seniors and may be graduated in January, 1918.

*Note.*—In taking this step, not only the military demands upon the medical profession, but civil demands as well are taken into consideration.

3. Taking into consideration the future needs of the country for trained medical men, it is the opinion of the Faculty of the University of Michigan Medical School that it is advisable for the undergraduate medical students to complete their course of instruction and not to enlist.

4. The Faculty of the University of Michigan Medical School recommends that not less than two hours per week be set aside for the military drill of undergraduate students, and that in addition to the ordinary infantry drill, we recommend training along the lines developed by the Clinical Society of Albany, and known as the "Albany Plan."

*Note.*—The medical officer should first of all be a soldier. This is necessary in order to make him most efficient as a medical officer.

5. That copies of these resolutions be furnished for suggestions of approval or disapproval to the following bodies:

- (1) The Surgeons General of the Army and Navy.
- (2) The National Medical Committee on Preparedness.
- (3) The National Research Council.
- (4) The Faculties of other medical schools.

6. That a list of the graduates of the classes of 1915 and 1916, with their standing while in the school and their present addresses, be sent immediately to the Surgeons General of the Army and Navy.

In compliance with the above resolutions I am hereby submitting them to you, and will be grateful for immediate expressions of opinion concerning them.

I have the honor to be, yours most respectfully,

VICTOR C. VAUGHAN, DEAN,  
University of Michigan Medical School.

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### *The Toxemias of Pregnancy*

THE strain to which the metabolic functions of the maternal organism is subjected in pregnancy is frequently so great that toxic substances accumulate to such an extent as to produce symptoms not infrequently ending in death. In the earlier months these manifest themselves on the digestive functions, producing the vomiting of pregnancy, a symptom that is so common as to be considered as a more or less normal sign of the condition. Sometimes, however, the toxemia even at this stage is so severe that the vomiting becomes intractable—the so-called pernicious vomiting. Later in pregnancy the maternal organism, as a rule, develops some means of defense against the toxemia, and the digestive symptoms disappear; but sometimes this defensive mechanism seems suddenly to break down so that the toxic symptoms again assert themselves, affecting now, however, not so much the digestive apparatus as the nerve centers, and thus producing

eclamptic convulsions. The defensive mechanism against the development of these later symptoms seems to break down often with great suddenness. A case that is apparently running a perfectly normal course may unexpectedly develop eclampsia, although usually the accumulation of the toxic substances in the mother manifests itself by the gradual rise in diastolic pressure and often by the presence of albumin in the urine. Such a picture of the functional pathology of the toxemias of pregnancy may not at first seem to fit in with all the observed facts, but it does so closely enough to make it serve as a useful hypothesis from which to attack this most difficult problem.

The first question that presents itself concerns the nature of the toxic substance. According to the above hypothesis, this toxin acts over a long period of time; its action, in other words, is a cumulative one, so that it is not surprising to find that only negative results are obtained when an attempt is made to cause the symptoms to develop in a normal animal into which the blood of an eclamptic patient is injected. If positive results could be obtained in such an experiment, it would be rational to attempt the isolation of the toxic substance from the maternal blood, but since this is not the case, the only procedure open to us lies in patiently searching for possible metabolic abnormalities in the maternal organism. Three of these have recently received a considerable degree of attention; namely, a faulty metabolism of the amino acids; an improper relationship between ammonia and urea; and an increase in the H-ion concentration of the blood—in other words, a condition of acidosis. Losee and Van Slyke have recently contributed a most important article bearing on these possibilities. With regard to amino acid metabolism, they found, in twenty-three cases, that the amino nitrogen of the urine did not exceed the amount present in normal individuals (i. e., 3.6 per cent of the total N) except in three of the cases, but even in these the increase was not beyond that found in normal pregnancy, where there is a tendency for the amino nitrogen to be a little above the physiological level.

Since it is possible that a failure in amino acid metabolism might exist without going so far as to appreciably affect the amino acid content of the urine, the blood was also examined for an accumulation of these bodies, and again with negative result. "Not a single one of the ten eclamptic women whose blood was examined for amino nitrogen showed a figure outside the range of 4-8 mg. per 100 c.c. noted . . . in a series of normal men." Quite clearly, then, faulty metabolism of amino acids is not responsible for the toxemia of pregnancy, and the question naturally arises as to whether the excretion of the end products of protein metabolism, namely, ammonia and urea, might not be abnormal. The urea of the blood and urine and the ammonia of the urine were therefore determined, and with significant results. Thus, the urea of the urine, in relationship to the total nitrogen, was usually below the average normal; indeed, except in cases in which the examination was made one or more days *post partum*, it was below 70 per cent of the total nitrogen, values as low as about 50 per cent being obtained in a considerable number of the cases. The relative amount of ammonia was also higher than normal, although not so strikingly so as that of the urea. Very high ammonia figures were, however, obtained in six cases of pernicious vomiting, namely, always above 16.9 and sometimes as high as 30, the figures for normal men being usually given as varying between 3 and 11 per cent.

according to diet. The relatively high ammonia excretion may in part be due to the influence of starvation which in itself is well known to raise the ammonia ratio; but even admitting this, there can be no doubt that considerable diagnostic value attaches to an observation of the ammonia. It is pointed out that the relatively low urea and high ammonia is suggestive of the condition found by Nencki and Pavlov in the urine of dogs in which the liver had been partly removed from the circulation by the establishment of the Eck fistula. It is of further significance that such animals often suddenly develop symptoms which have some similarity to those of eclampsia, especially when the animals are fed exclusively on meat.

To investigate the possibility that acidosis might be responsible for the symptoms, Losee and Van Slyke examined the carbon dioxide combining power of the plasma by the method already described by them.<sup>1</sup> Sufficient results have now been collected to permit us to state that the blood of normal persons does not take up less than 55 c.c. nor more than 75 c.c.  $\text{CO}_2$  per 100 c.c. of plasma; still narrower normal limits, but at a somewhat higher level, being possible when the method described by Austin and Jonas<sup>2</sup> is employed. The general average may be taken as 65 c.c.  $\text{CO}_2$  per 100 c.c. plasma. Using the  $\text{CO}_2$  tension of alveolar air as an indicator Hasselbalch and Gammeltoft have shown that a slight degree of acidosis exists in normal pregnancy. Values below the normal minimum (55) were observed by Losee and Van Slyke on at least one day in ten of fourteen cases of pregnancy examined. In cases of toxemia, whether of the eclamptic or vomiting type, no greater a degree of acidosis than that present in normal pregnancy was observed to occur. The  $\text{CO}_2$  combining power of the plasma never approached anywhere near the low values of 30 c.c.  $\text{CO}_2$  per 100 c.c. of plasma found to be present in cases of typical acidosis as met with in diabetes and nephritis.

The great importance of this investigation lies in the perfectly definite and final evidence which it supplies that neither faulty metabolism of the amino acids nor a condition of acidosis is accountable for the symptoms of the toxemia of pregnancy. The results indicate, however, that something is radically wrong with that stage in the metabolism of nitrogen which is responsible for the final balance between the urea and ammonia excretions. One other very important outcome of the investigation goes to show that there is not necessarily any relationship between the ammonia excretion and the degree of acidosis. Thus, in the cases of pernicious vomiting where the  $\text{NH}_3$  excretion was abnormally high, the plasma did not absorb any less  $\text{CO}_2$  than that found in cases of normal pregnancy.

<sup>1</sup>Losee, J. R., and Van Slyke, D. D.: The Toxemias of Pregnancy, *Am. Jour. Med. Sc.*, 1917, cliii, 94.

<sup>2</sup>The modification referred to consists in drawing the blood directly from the patient's vein through tubing, which passes to the bottom of a centrifuge tube containing some oxalate crystals and paraffin oil. The latter floats on the surface of the blood and thus prevents loss of  $\text{CO}_2$  during the centrifuging. The experimental error can be still further cut down by saturating the oxalated blood with  $\text{CO}_2$  at 6 per cent of an atmosphere. The rationale for these precautions is that the  $\text{CO}_2$  combining power of plasma is influenced by the  $\text{CO}_2$  tension of the blood existing at the time when the corpuscles and plasma are separated. Austin, J. H., and Jonas, L.: *Clinical Studies in Acidosis*, *Am. Jour. Med. Sc.*, 1917, cliii, 81.

## Hodgkin's Disease

FOR about eight years Bunting and Yates have been engaged in an intensive study of Hodgkin's disease, which they believe is an infectious disease caused by an organism, *B. hodgei*,<sup>1, 2, 3</sup> a pleomorphic diphtheroid, which stains by Gram's method. Beside being an infectious disease, the complex has also the destructive potentialities of the malignant neoplasm, and these two facts have been fundamental in the method of treatment which these two workers have perfected.

For the purpose of comparing the effects of treatment in different cases Bunting and Yates<sup>4</sup> have grouped them, using a classification which is an amplification of Trousseau's earlier one. Trousseau classified Hodgkin's disease into latent, progressive, and cachectic cases. Bunting and Yates' modification is as follows (the schema indicates the number of cases they report):

All cases (63)	{	Acute cases (5)		Group 1		5 cases
		{	Stage 1 (8)	{	Early	Group 2
	Late				Group 3	3 "
	Stage 2 (25)		{	Early	Group 4	11 "
				Late	Group 5	14 "
	Stage 3 (25)		{	Early	Group 6	14 "
				Late	Group 7	11 "

In the acute cases the blood picture,<sup>5</sup> the most reliable single diagnostic evidence, is often obscured by the leucocytosis. "It is fortunate," say the authors, "that the incidence of this form of the disease is comparatively slight, for it is about as amenable to treatment as is the mastitis carcinomatosa of lactation or melanoma."

Group 2, composed of *incipient cases*, has a blood picture that is characteristic, and this picture appears early. In these cases the portal of entry has been recently established and only a few adjacent glands are beginning to be palpable. The only difficulty is that the diagnosis at this stage is fortuitous and depends largely upon careful physical examination, routine blood counting, and accurate interpretation of tuberculin tests.

Group 3, in which the *early cases* are placed, has the characteristic blood picture. In these there is unmistakable involvement of a primary group of glands but without demonstrable extension. In this group and every other more advanced one the occurrence of a specific bacteriemia of some extent is virtually certain at one or more of the positive phases.

Group 4. In this group are the *moderately advanced cases* in which the blood picture is positive but not of an early type. In them more than one of the main superficial gland groups are involved but extension to the mediastinal or peritoneal glands can not be more than suspected.

Group 5. In these, the *advanced cases*, the blood picture may be like

<sup>1</sup>Bunting and Yates: Jour. Am. Med. Assn., 1913, Ixi, 1803; Ibid., 1914, Ixi, 516.

<sup>2</sup>Bunting, Yates, and Kristjanson: Ibid., 1914, Ixiii, 2225.

<sup>3</sup>Bunting and Yates: Arch. Int. Med., Aug., 1913.

<sup>4</sup>Bunting and Yates: Jour. Am. Med. Assn., 1917, Ixviii, 747.

<sup>5</sup>Bunting: Bull. Johns Hopkins Hosp., 1911, xxii, 248.

that of Group 4, or it may show a reduction in nearly all the cell forms. In them there is always demonstrable involvement of deep structures.

Group 6 is composed of *cases which are probably hopeless, but capable of palliation*. The blood picture is of the late type.

Group 7 is made up of *cases with lethal involvement and probably beyond palliation*.

The treatment used by Bunting and Yates is based upon the belief that the disease is a chronic infection of lymphoid tissue, and that there is evidence that the affected persons are incapable of a permanent protective reaction without assistance. They therefore attempt to assist the patient by thorough excision of as much infected tissue as possible; to remove or heal possible portals of entry (tonsils, teeth, dermatitides, bronchitides, etc.); to add to the patient's resistance by the use of immune sera; and to reduce or remove as much potential or existing lymphoid tissue as may be possible by the use of the x-ray.

The results attained are of exceeding interest when taken in conjunction with the previous mortality expectancy figure of 100 per cent.

Group 1.	Estimated possibility of recovery, less than	5%
" 2.	" " " "	from 80 to 90%
" 3.	" " " "	from 60 " 70%
" 4.	" " " "	from 30 " 40%
" 5.	" " " "	from 5 " 10%
" 6.	" mortality	100%
" 7.	" "	100%

These figures mean that in its early stages Hodgkin's disease is a very curable disease, in which respect it is quite like carcinoma. In each case early recognition demands patient care.

—P. G. W.



# *The Journal of Laboratory and Clinical Medicine*

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ST. LOUIS, MAY, 1917

NO. 8.

## *ORIGINAL ARTICLES*

### *CASES OF UNUSUAL ANEURISMS\**

BY PAUL G. WOOLLEY, M.D., CINCINNATI, OHIO.

ANEURISMS are most frequently encountered in connection with aortic lesions, especially syphilitic ones, and are less frequent occurrences in the other vessels of the body, with the possible exception of the smaller arteries of the brain. Also, with the exception of traumatically produced ones, and an occasional one resulting from pure atheroma, they are the consequences of infection of the arterial walls. Even in the cases of pure atheroma there is a question whether or not that process is not fundamentally infectious. Aneurisms appear at sites of high arterial pressure and in situations where the arterial walls are weakened to a degree which permits of "giving way." In the aorta and in the cerebral circulation increases of blood pressure are less easily accommodated, other things being equal, than in the peripheral vessels where a larger area is available for taking care of increased pressures. Also in the peripheral vessels the arteriosclerotic processes are more apt to be diffuse, less apt to be focal, and therefore the common tendency to form a saccular aneurism is absent. There are, however, certain instances in which arteries of moderate dimensions are focally damaged to such a degree that a saccular aneurism develops. In some such cases the cause lies in some lesion outside the vessel, but gradually encroaches upon it until it is weakened and bulges. Such a process is common in tuberculosis. In such cases the vessel wall becomes involved in a tuberculous process which leads to focal dilatation, and sometimes rupture and hemorrhage. There are other cases in which the aneurism appears to be due entirely to a process which commences in the arterial wall itself, a process which is essentially the same as that appearing in the aorta in cases of aneurism. Next to the aorta, the popliteals, femorals, carotids, subclavians and innominate are the com-

\*From the Mary M. Emery Department of Pathology of the University of Cincinnati, and the Pathologic Institute of the Cincinnati General Hospital.

mon seats of spontaneous aneurisms. Other vessels are less frequently affected.<sup>1</sup> The following statistics indicate the location of aneurisms in 530 cases tabulated by Crisp.<sup>2</sup>

Thoracic aorta .....	175
Popliteal artery .....	137
Femoral artery .....	66
Abdominal aorta .....	59
Carotid artery .....	25
Subclavian artery .....	23
Axillary artery .....	18
External iliac artery.....	9
Cerebral artery .....	7
Common iliac artery.....	2
Posterior tibial artery.....	2
Gluteal artery .....	2
Pulmonary artery .....	2
Brachial artery .....	1
Subscapular artery .....	1
Ophthalmic artery .....	1

The following series of cases illustrates these more unusual types of aneurism.

#### CASE I.

Not infrequently the innominate artery is involved in dilatations affecting the contiguous parts of the aortic arch. Less commonly the innominate artery alone is the source of the condition. The following case illustrates the latter circumstance:

J. K., Hospital No. A-1400, a colored man 51 years old, was admitted to the Cincinnati General Hospital on Feb. 25, 1916. He died two days later. He complained of shortness of breath. The family history was negative.

PAST HISTORY.—He had had measles, smallpox and chills and fever. He denied gonorrhea, but admitted lues. Except for these illnesses he had been a healthy man.

PRESENT ILLNESS.—On March 18, 1915, the patient was at work when he felt a pain in his shoulder, upon which he could feel a little "knot." He says that on that day he had carried a pine board over his shoulder and this he thought accounted for the pain. At this time he could not open or close his right hand. He went to a physician who told him he had an aneurism. Later he went first to one hospital and then to another and then, finally, he came to the Cincinnati General Hospital. Since then he has been admitted twice. He says that he has a cold sweat on the left side and that the left side of his throat feels cold and that he has a sensation as though phlegm is in his throat. He is dyspneic and does not speak easily.

PHYSICAL CONDITION.—The patient is a well developed, somewhat emaciated colored man about fifty years old, sitting in bed bent forward with his arms on his knees. He has marked inspiratory and expiratory dyspnea, with a harsh brassy sound. He coughs frequently, and the cough has a very metallic ring. The voice is likewise harsh and metallic. The left pupil is larger than the right. Both pupils are regular in outline. The left reacts to light and accommodation, the right slightly, if at all, to light. The conjunctivæ are pale. The teeth are in fair condition. There is some pyorrhea. The tongue is furrowed in the center and is covered with a thick yellowish coat. The throat is moderately injected. The tonsils are not enlarged. (During this part of examination the patient asked for water which he had a great deal of difficulty in swallowing. None was regurgitated through the nose.)

The veins over the right arm and chest anteriorly and the veins in the right side of the neck are dilated. The base of the neck on the right side at the right supraclavicular space is prominent, more so at the sternal end. On palpation there is a hard mass occupying this space over which the skin is freely movable. This mass extends over the junction of the outer and middle third of the clavicle to the left border of the supra-

sternal space. It is apparently continuous with the right carotid and right supraclavicular arteries. It pulsates visibly and palpably, raising the clavicle with each pulsation. No thrill can be felt. The chest is somewhat thin; the supra- and infra-clavicular spaces and also the intercostal spaces are marked on both sides. There is retraction of the left apex and all of the intercostal spaces and epigastrium. During inspiration the respiratory excursion is apparently equal on the two sides. On percussion over the right supraclavicular space there is marked absolute dullness over the mass described above. This dullness extends to the 2nd rib. Below this the percussion note on the right seems slightly higher pitched and shorter in duration than that on the left which is hyperresonant. The lower lung border on the right is at the 6th rib in the midclavicular line. Posteriorly a faint pulsation can be felt at the upper part of the supraspinous fossa on the right. Above the level of the spine of the scapula on the right percussion is less resonant than on the left. Below this the percussion tones are about equal on the two sides, and somewhat hyperresonant. The lung borders below are at the level of the 12th dorsal spine. Below this on each side there is about an inch of impaired resonance and then tympany extending down to the sacrum. On auscultation the breath sounds on both sides of the chest anteriorly and posteriorly are very distant. Inspiratory and expiratory sounds in the trachea are transmitted over the whole chest, interfering with the satisfactory auscultation.

The apex beat is seen in the 6th interspace 10.5 cm. to the left. Relative cardiac dullness, 12.5 cm. to the left in the 6th; 11.5 cm. to the left in the 5th; 7.5 cm. in the 4th; 3.5 cm. in the 3rd. Retrosternal dullness 7.75 cm. broad. Retrosternal dullness on the right is slightly greater than the dull area described above as extending down over the clavicle to the 2nd rib. With the patient in a sitting position the abdomen is lower than chest. Liver dullness extends about 3 fingerbreadths below the costal margin. The spleen is not felt. There is retraction of the abdomen on inspiration and bulging on expiration, especially in the epigastrium. No rigidity. No hernia. No masses and no tenderness palpable. Extreme knee kicks present. There are scars on both shins. There has been apparent recent loss of flesh.

The hands are cold. The pulse is scarcely palpable in the right brachial artery, and is absent at the radial, though well marked at the left wrist. The right arm is larger than the left and is apparently the seat of a very soft edema which pits only very slightly.

On auscultation over the apex the heart sounds are rather weak though clear. No murmurs are heard. At the base the sounds are still weaker. The pulmonic second is soft. The aortic second is almost inaudible. There are no murmurs over the aortic area. Over the mass in the neck the heart sounds were heard superimposed upon a sort of continuous roaring noise not definitely related to any part of the cardiac cycle. The blood pressure was the same in each arm, namely, 190 systolic, 110 diastolic.

Urine, Feb. 26, 1916. Amber, acid, sp. gr. 1.024; albumin present, no casts, few leucocytes, much cellular debris.

X-ray report February 26th. X-ray plate of chest shows a large opaque mass springing from the upper part of the mediastinal shadow and extending up into the right apex. The shadow is about the size of a large grape fruit, has rounded borders and is of uniform density. The heart outline is negative except for some accentuation of the upper left aortic curve. Lung areas and diaphragm contours are negative.

The temperature on admission was 99.6°, ranging between 100.4° and 97.6°. Pulse between 122 and 68. Respirations 30 to 38.

The patient had continuous difficulty in breathing and gradually grew weaker. He died quietly, apparently of exhaustion, at 5:45 A.M., Feb. 27, 1916.

CLINICAL DIAGNOSIS.—Syphilis; aneurism of the transverse arch of the aorta including the innominate artery; hypertrophy and dilatation of the heart; syphilitic aortitis; chronic diffuse nephritis; arteriosclerosis; congestion of the viscera; aortic and mitral regurgitation; chronic bronchitis; kyphosis; emphysema.

#### AUTOPSY PROTOCOL (C).

The body was that of a well developed, well nourished colored man of about 45 years of age, with a general glandular enlargement. The right suprasternal region was fuller than the left. The veins of the right arm were distended and engorged. The right pupil was larger than the left. The teeth were for the most part in fair condition. The gums were retracted and pyorrheic.

On opening the thorax, the lungs collapsed. The right lung was free. The left was bound down by firm fibrous adhesions to the lateral surface of the lower lobe. There was a mass in the superior mediastinum measuring 8 cm. in diameter, the upper limit of which was 3 cm. above the clavicle on the right side. The right clavicle was eroded at the sternoclavicular joint. The right lung was voluminous, crepitated throughout, and felt and looked normal. The left lung crepitated throughout the upper end, to a less extent, the lower lobe. The lower lobe, on section, was dark red in color, thickened, and from it a frothy, sanguinous material could be expressed.

The heart was enlarged and dilated, especially on the right side. The right auricle was filled with a chicken-fat clot. The tricuspid orifice admitted three fingers with ease. The tricuspid and pulmonary valves appeared healthy. The mitral orifice admitted two fingers. The leaflets of the mitral valve, as well as those of the aortic, showed no abnormal change. The papillary muscles cut with slightly increased resistance and were streaked with fibrous tissue, especially at the apices. The mural myocardium presented a slight increase in fibrous tissue. The entire myocardium was pale. The aorta showed scattered patches of fatty degeneration, but no sclerosis, or thickening. The mouths of the coronaries were patent. There was a sacculated aneurism of the innominate artery measuring 5 cm. in its greatest diameter, filled with laminated blood clot partially organized. This aneurism arose from the innominate artery about 3 cm. above its origin, and had an orifice 1 cm. in diameter.

The liver was of about normal size, was mottled yellow and purple, purple predominating. The capsule was not thickened, although there were a few stellate scars upon it which did not extend into the organ. On section, the interlobular veins were injected. There was a slight increase of fibrous tissue and fat. The spleen was very much larger than normal, was grayish-red in color. On section, the pulp was of a very dark color, the Malpighian bodies were visible though not increased. The right kidney was slightly smaller than normal, was pale red in color, the capsule stripped with ease leaving a granular surface. On section, the glomeruli were visible, the relation between cortex and medulla was normal, the line of demarcation was not distinct. The left kidney resembled the right in all respects.

The stomach, intestinal tract, pancreas, and bladder, except for a slight congestion, showed no abnormalities. The prostate presented a slightly enlarged median lobe which bulged into the bladder.

ANATOMIC DIAGNOSIS.—Innominate aneurism; cardiac hypertrophy and dilatation; chronic diffuse nephritis (interstitial); general lymphadenoid hyperplasia; chronic passive congestion of the liver, spleen and intestinal tract; prostatic hypertrophy.

#### REMARKS.

This is an interesting case from the standpoint of the arterial system generally. There was a history of syphilis, and there was an aneurism; there was accordingly a good chance that in the aorta at least there would be evidence of the infection. That there was no such evidence is unusual. This makes one wonder whether the aneurism was not therefore really a traumatic one, in spite of the fact that it would be difficult to strike the innominate 3 cm. from its origin. Still, stranger things have happened! The only evidence of lues in the body was furnished by the stellate liver scars—and they were not brilliant. Finally it occurs to one,—Morris suggested it,—that under the circumstances this case would have justified surgical treatment. Also it is interesting that aneurism localized in the innominate artery is not mentioned in Crisp's statistics.

#### CASE II.

This case is one in which the aneurism was localized in the superior mesenteric artery.

C. R., Hospital No. A-6412, a negro 25 years old, was admitted to the Cincinnati General Hospital on Sept. 25, 1916, complaining of pain in the belly, loss of weight, and

a mass in the abdomen. He died on October 9th. The patient had been in the hospital before. The following is the abstract of the previous history:

He was admitted on August 8, 1916, complaining of pain in the left side of the abdomen. This pain appeared suddenly while he was lifting and was just to the left of the midline and extended to the root of the penis. It was a sharp, continuous pain which did not radiate and which was relieved to some extent after bowel movements or urination. He had vomited once on the day before admission. He urinates frequently but has difficulty in starting the stream. Physical examination showed some emaciation, notched teeth; large epitrochlear axillary glands. There was slight dullness and bronchovesicular breathing over the upper lobe of the right lung. The heart examination was negative. In the upper abdomen, in the midline, or just a little to the left, was a mass which extended from xiphoid to umbilicus, and which was movable, not affected by respiration and tender. Over it there was tympany. The urine contained blood. The stools were normal. Cystoscopic examination showed both ureters excreting clear urine. The right was entered with trauma; in the left the catheter could not be passed. The bladder was normal. X-ray plates of the stomach were negative. Operation was suggested to the patient and refused by him.

The symptoms on readmission were the same. Physical examination showed that, as the patient had noticed, the abdominal mass was larger than before. This time the urine was clear and contained no blood or albumin. On September 30, an exploratory operation was done concerning which the following note was made:

"At the root of the omentum, surrounded by adhesions, a mass was found about the size of a small muskmelon, globular in shape but extending up under the stomach. It was covered with peritoneum. The abdominal aorta could be palpated posteriorly. The kidneys on either side were apparently normal. A fine needle was introduced and about 10 c.c. of a fluid withdrawn that was apparently blood. A large trocar with tubing attached was introduced and 20 oz. of fluid withdrawn. This fluid seemed to be almost pure blood but there was a cloudiness to it as it ran out as if mixed with a little pus. The trocar hole oozed a little blood after the withdrawal of the trocar but with a little pressure the bleeding soon stopped. A fold of omentum was stitched down with silk over the surface of the tumor where it had been punctured."

*September 30.*—The patient, after returning to ward had a very weak rapid pulse but was quiet and did not complain much of pain. Later he hiccupped and vomited a good deal and was quieted by morphine.

*October 1.*—The condition very little improved; pulse weak and temperature subnormal.

*October 3.*—The patient seems a little better; complains of pain in upper abdomen.

*October 6.*—The patient in a stupor; pulse good.

*October 7.*—Still in stupor, breathing deeply, pulse strong.

*October 9.*—Stopped breathing suddenly at 10:10.

CLINICAL DIAGNOSIS.—Aneurism of the abdominal aorta; syphilis; generalized arteriosclerosis.

#### AUTOPSY PROTOCOL (G.-M.).

The body was that of a poorly nourished negro, about the age of 45 apparently, and about 5 feet 10 inches tall. The pupils were equal and not dilated. Arcus senilis was present. The eyeballs were sunken and the sclerae discolored but not jaundiced. Rigor mortis was present; postmortem lividity was present in the dependent portions. The teeth were in poor condition and cavities were present especially on the anterior surfaces of the incisors. Pyorrhea was present.

There was a transverse incision in the abdomen extending from the left nipple line to 2 inches beyond the right nipple line with the convexity pointing upward, the highest point being  $2\frac{1}{2}$  inches above the umbilicus. The incision was closed with catgut and seemed to be about eight days old. The tissues beneath were infiltrated with a dark blood and the omentum was adherent. In the left upper quadrant a mass was found 1 inch below the umbilicus and extending up to the costal margin. The omentum was adherent to this mass, which was in turn adherent to the stomach. The whole mass with the surrounding organs was removed and dissected. It measured, after dissection,  $6 \times 5 \times 5$  inches, and was adherent to the pyloric portion of the stomach, the duodenum, the ascending and transverse colon, and the lower surface of the left lobe of the liver. Behind it

was the abdominal aorta which also was adherent. The abdominal aorta was opened and then it was found that from this vessel an artery corresponding in position to the superior mesenteric passed into the tumor, which on being opened showed that it was an aneurismal sac due to dilatation of the artery. The sac was almost completely filled with blood clot, but at the proximal part contained a couple of ounces of blood. The intestines were congested and the urinary bladder extended about 1 inch above the symphysis pubis. The lower border of the liver was 2 cm. below the ensiform, and about level with the costal margin in the right mammillary line. The appendix was *in situ* and normal in appearance.

When the thorax was opened, the lungs collapsed and there was no fluid in either pleural cavity. On the right side adhesions were found at the lower border of the upper lobe and the anterior upper and outer surfaces. There were a few adhesions at the apex and adhesions were found between the lobes. The left lung showed lateral and apical adhesions. There was no increase in pericardial fluid. The left kidney was flabby, purplish-red in color, and on cross section was pale and the cortex thinned. The substance was very firm to the feel and the pyramids appeared fibrous. The glomeruli were visible as fine points. The capsule stripped with difficulty, was very thin, and on removal, tore the kidney substance in spots. The stellate veins were slightly congested. The pelvis was normal. The right kidney was similar. The liver was of normal size, with the scars of old adhesions over its surface. In the capsule there were numerous fine white lines that radiated from a number of points. At various points over the whole organ, stellate depressions and scars, due possibly to healed syphilitic gummata, were found. These extended into the substance of the liver at variable depths, the deepest being about 3 mm. Similar ones were found also in the substance. On cross section, the liver was pale pink in color, the interlobular vessels were readily apparent and the liver showed a slight yellowish tint. The bile ducts contained an unusual amount of bile and the organ had a firm feel. The gall bladder was distended, the wall slightly thickened and contained about 3 ounces of dark blackish mucoid fluid. The spleen was of normal size and the capsule about normal. On section the pulp was found to be very firm to the feel. The blood vessels were enlarged and stood out. There was an increase of fibrous tissue. The apex of each lung showed scars of old adhesions. All the lobes were bound together. The lungs were voluminous and crepitated throughout. On cross section, a frothy, blood fluid was exuded, which was greatest in the upper lobes.

The heart was slightly enlarged and the right side was dilated. The valves were healthy. The internal surface of the aorta showed a number of fatty plaques and in the descending portion below the origin of the transverse arch there were areas of sub-intimal thickening resembling luetic changes. The coronaries were not tortuous. The myocardium was pale and there was no macroscopic evidence of fibrosis.

**ANATOMIC DIAGNOSIS.**—Aneurism of the superior mesenteric artery; gumma of the liver; hepatic capsular fibrosis; luetic mesaortitis; generalized arteriosclerosis; splenic fibrosis; chronic diffuse nephritis and passive congestion.

#### REMARKS.

How infrequent superior mesenteric aneurisms are may be seen from von Schrötter's<sup>3</sup> figures as given by Lang.<sup>4</sup> In 19,300 autopsies an aneurism was found in connection with the superior mesenteric artery in but one instance, whereas there were four splenic aneurisms, one celiac, one renal, and three of the abdominal aorta. In the total number of autopsies 220 aneurisms were encountered.

In recent medical literature I have found several cases of mesenteric aneurisms, but all have been either traumatic or definitely mycotic and acute. Such are the cases of Gifford<sup>5</sup> in which there was a septic endocarditis, Rolleston and Whipham,<sup>6</sup> Cotterill and Miller,<sup>7</sup> and Stern.<sup>8</sup>

#### CASE III.

This is a case in which there was an almost extreme grade of generalized arteriosclerosis and in which a small aneurism was found in connection with the

almost completely calcified splenic artery. The case history is interesting chiefly on account of the symptoms associated with the general arteriosclerosis. It may be, however, that certain of the dominant clinical features had to do essentially with the splanchnic sclerosis in which the splenic artery was preeminently effected.

K. L., Hospital No. 6312, a white woman 63 years of age, was admitted to the Cincinnati General Hospital on December 1, 1915, complaining of "shortness of breath, heart trouble, lump in the stomach, and swelling of the right foot."

FAMILY HISTORY.—Her mother died at the age of 64 of "kidney trouble." Her father died at 63 of "abscess of the lungs."

PAST HISTORY.—She had measles, scarlet fever, and whooping cough in childhood. Ten years ago she had inflammatory rheumatism. Thirty years ago she had an ulcer over the left tibia. Sixteen years ago she had prolapsus uteri. She drinks tea to excess, but uses no alcohol.

PRESENT ILLNESS.—Last May the patient began to be short of breath on exertion. Her feet began to swell, the right more than the left. Later the abdomen became heavy and distended. The swelling gradually subsided. At the time of the onset she noticed two lumps, one on each side of the neck. The one on the left side comes and goes. For the last three months the patient has suffered with "indigestion," i.e., pain and distress after eating. She does not vomit, but belches and has a sour bitter taste in the mouth. For the last few mornings she has vomited stringy mucus which relieved the symptoms.

PRESENT CONDITION.—The patient is a poorly nourished woman. Her pupils react to light and accommodation. The conjunctivæ are slightly jaundiced; the eyelids are puffy. Both lids droop, the right more than the left. The teeth are poor. The gums are pyorrhæic. The external carotids present two expansile enlargements, the one on the left being higher. These pulsating "kernels" are visible and compressible and appear to be aneurisms. The brachial and axillary arteries are tremendously sclerotic and tortuous and have a varicose feel that suggests focal dilatations.

The skin over the chest is dry, scaly, and lemon yellow in color. There is no tracheal tug. The apex is diffuse in the sixth and seventh interspaces outside the nipple line, lifting the left side of the chest.

The vocal fremitus is increased on the right. Posteriorly the right side shows tubular breathing, roughened at times with scattered mucous râles. On the left side there is roughened inspiration, tubular blowing expiration at the base and, in the axilla, tubular breathing. Anteriorly on both sides there is a tubular quality more pronounced on the right than on the left. There are no râles or friction rubs. On percussion the resonance is impaired over both apices posteriorly. The left was almost flat; the right was dull. Over the scapula on the right dullness runs off into a dull hyperresonance in the axilla and base. On the left the apical dullness was continuous with impairment over the scapula and upper axilla while the base and lower axilla show hyperresonance. Anteriorly under the clavicle on the right and left there is dullness fading into dull tympanites on the right, and impaired resonance in the left axilla.

There is a wide increase of cardiac dullness as follows:

To right	To left
4	5
4½	5
4½	9
4½	11½
5½	12
6	12½

On auscultation a systolic bruit is well heard at the apex and also at the aortic area. The second aortic is much accentuated and doubled at times. At times the bruit seems to be between the first and second sounds and not connected with them. At each beat there is a tremendous upheaval of the left chest. Blood pressure systolic, 240; diastolic, 145.

The abdomen is relaxed. There are no masses and no abnormal pulsation. There

is epigastric tenderness. The liver margin is made out 2 fingerbreadths below the costal margin. There is no hepatic pulsation.

Upon the extremities the veins are varicose, tortuous and knotty. There is evidence of old varicose ulcers. The reflexes are normal.

*December 3.*—The patient complains of a smothering sensation and of palpitation. She is conscious of violent heart action. The urine shows a trace of albumin and casts.

*December 4.*—An x-ray plate shows curvature of the spine and some displacement of the heart to the left. Just to the right of the aortic arch there is a large calcified gland. A further note on the plate says that the heart is enormously enlarged to the right, left and downward. There is an old fibroid condition at both apices. Very little lung is left on the left side.

*December 5.*—Urine excretion poor. Beginning edema of the lungs.

*December 6.*—Increased edema of the lungs. Patient lapses into unconsciousness. Pulse 120. Blood pressure, systolic, 200; diastolic, 120. Appearance of a petechial eruption. At noon, blood pressure, systolic, 180; diastolic, 90. Later Cheyne-Stokes respiration developed, and after remaining semicomatose until the following day at 5:15 P.M., the patient died.

CLINICAL DIAGNOSIS.—Mitral insufficiency; chronic nephritis; cardio-vascular-renal sclerosis; arteriosclerosis; varicose veins; hypertrophy of the left ventricle; edema of the lungs; anasarca; fibroid pulmonary tuberculosis; calcified mediastinal glands; possible aneurism of the thoracic aorta; passive congestion of the liver and spleen; chronic gastritis.

#### AUTOPSY PROTOCOL.

The body of an old, gray-haired, slenderly built, thin, woman of about 60 years. The abdomen seemed a little distended. The peripheral venous system was congested, so that the superficial vessels of the thighs, upper arms, thorax and legs showed very distinctly through the pale skin. There were well marked varicose veins in each leg, especially the left, and here the skin was generally thickened and scarred as though it had been frequently ulcerated. Both legs were slightly edematous. Posterior lividity was exceedingly brilliant, but it was patchy rather than diffuse, and appeared to be composed of numerous cutaneous hemorrhages closely set together. Both buttocks were mildly excoriated. The finger nails were dusky. The subcutaneous fat was scanty. The mammary glands were atrophic, but normal.

When the body was opened, the lungs did not collapse, because of a moderate edema and mild hypostatic congestion. There were adhesions at both apices. The liver border was 11 cm. below the ensiform and 8 cm. below the costal margin in the right mammillary line. The intestines were in normal position except the transverse colon which was very low and filled with large firm scybala. Except for the presence of two fibromata, the pelvic organs appeared normal.

The lungs (left 330, right 675 grams) contained just enough fluid and blood to keep them from collapsing. In each apex was a mass of calcium-containing fibroid tissue. They were congested posteriorly.

The heart (570 grams) was large and firm. The right auricle was tremendously dilated and filled with a large chicken-fat clot. The foramen ovale was closed. The tricuspid orifice admitted three and a half fingers. The valves were healthy. The pulmonary leaflets were healthy and the orifice normal. The mitral orifice admitted two and a half fingers. The leaflets were healthy except for a moderate edema of the margins. The aortic orifice was not dilated (1+ fingers). The leaflets were comparatively healthy. The line of attachment was a little sclerotic, and at the points of contact there was some adhesion. The free margins were thin. The myocardium was tremendously thickened, and the ventricular cavities showed practically no dilatation. The coronaries were not sclerosed. The aorta was the seat of a general atherosclerosis which was least developed in the arch and most in the abdominal segment. As a matter of fact the sclerosis was most intense, with the exception of the midabdominal section, in the main branches of the aorta, the iliacs, the celiac axis and its branches, the renals and the carotids, all of which were diffusely calcified and somewhat dilated. The dilatation in these vessels was more marked than in the aorta itself, where it was slight. The most strikingly changed vessel was the splenic artery which was exceedingly sclerotic and tortuous so that it looked like a large calcified twisted earthworm. At one point the vessel was dilated so that a small aneurismal sac the size of a small cherry appeared. Next in



intensity of involvement were the iliacs. The epicardium was thickened and scarred and the thickened areas were edematous.

The liver (1455 grams) was brownish in general color. The capsule was smooth and thin except for a patch about the size of one's palm on the anterior surface of the right lobe, where it was superficially thickened. The cut surface showed the early markings of passive congestion upon the yellowish brown substance.

The spleen (145 grams) was congested and deep purplish-red in color. The capsule was irregularly and superficially thickened. The substance was firm and in it the follicles could not be seen.

The pancreas showed no evident changes.

The kidneys (left 130, right 110 grams) were small. The capsules were removed with little difficulty though the granular surfaces were torn at a few places. The organs were generally mottled with red and yellow. They were evidently congested. The cut surfaces were firm and mottled. The cortices were thin.

The stomach contained a large amount of thin, bile-stained, cloudy fluid. The mucosa was congested and showed a few small pinpoint hemorrhages in the mucus-covered, slightly adherent mucosa. The duodenum was apparently healthy except for an increase in the mucus content. The bile ducts were patent. There were no gall stones in the gall bladder.

The uterus was small and atrophic. In the fundus was a large myoma, and near it to the left was a small one. The ovaries were sclerotic. There were no pelvic adhesions.

The urinary bladder and ureters were healthy.

**ANATOMIC DIAGNOSIS.**—Cardiac hypertrophy; dilatation of right auricle; aortic atherosclerosis; peripheral and splanchnic arteriosclerosis; arteriosclerotic kidney; obsolescent calcareous tuberculosis of lungs and bronchial lymph glands; fibromyomas of the uterus; varicose veins of legs; passive congestion of liver and spleen; chronic catarrhal gastritis; aneurism of the splenic artery.

#### REMARKS.

According to the figures of Crisp splenic aneurisms are very rare; according to those of von Schrötter, they are rare, but more frequently found than similar lesions of the renal or superior mesenteric arteries. Certain it is that in recent literature there are more references to splenic aneurisms than to most other forms of unusual abdominal aneurisms. In many instances the lesions appear to be due to embolic processes, as in the cases of Monroe,<sup>9</sup> and Walz.<sup>10</sup> In other instances, as in the present one, they appear to be definitely arteriosclerotic (luetic?) in origin.

#### CASE IV.

In this case the aneurism was associated with a congenital cardiac septal defect in a young infant.

D. B., Hospital No. A-7751, a white child 7 months old, was admitted to the Cincinnati General Hospital on November 22, 1916, for "shortness of breath."

There was no family history of tuberculosis or lues.

**PAST HISTORY.**—Except for an attack similar to that for which she was brought to the hospital, she has always been in apparently good health. She has been fed on Horlick's malted milk.

**PRESENT ILLNESS.**—This commenced five days before admission with cough and dyspnea.

**PHYSICAL EXAMINATION.**—The patient is a well developed white female infant seven months old, lying in bed crying and breathing rapidly and with difficulty. On admission the temperature was 100°, the pulse 120, and the respirations 38. The head is negative. The face is cyanotic and the respiratory movements are short and labored. The pupils are contracted and respond to light. The conjunctivæ are pale. There is no ocular deviation. The tongue is clean. The lower central incisor teeth are just emerging through the gums. The posterior pharynx is inflamed; there is a membrane. The nose is normal and there is no discharge from the ears. There is no rigidity of the neck. The finger

tips of both hands are cyanotic and the nail beds are deep blue. The chest is well formed and symmetrical. The respiratory rhythm is irregular and embarrassed. There is retraction of the peripneumonic groove during inspiration. There is dullness at the right base posteriorly, and crepitant and subcrepitant moist râles are heard throughout both lungs. Vocal fremitus is slightly increased at the right base posteriorly. The apices are clear. There is no distention or retraction of the abdomen. The liver dullness is normal. The spleen is not palpable. The genitourinary tract is negative. The toes of both feet are cyanotic. The apex beat is irregular and is visible in the 5th interspace inside the nipple line. The cardiac rhythm is regular. There is a harsh systolic bruit heard diffusely over the entire precordial region, but with a point of maximum intensity at the base, and apparently transmitted toward the apex, but not into the axilla.

*November 24.*—There is a distinct dullness over the right lower lobe from the angle of the scapula to the base. There is no dullness on the left side. Over the area of dullness there are crepitant and subcrepitant râles. Over the whole chest there are medium sized mucous râles. Anteriorly over the left upper lobe there is a breezy bronchial breathing without crepitant râles. On the right side the breathing is very rough.

*November 28.*—Over the right lower lobe there is still dullness but the râles are large and more moist. The general appearance is better. Respirations are not so labored.

*December 2.*—The condition is unchanged. Cyanosis is marked.

*December 3.*—The condition is unchanged. At 10:30 P.M. marked abdominal distention appeared and dyspnea and cyanosis increased.

*December 4.*—Death occurred at 8 A.M.

CLINICAL DIAGNOSIS.—Congenital stenosis of the pulmonary artery; patent foramen ovale; patent ductus arteriosus; bronchopneumonia.

#### AUTOPSY PROTOCOL.

The body was that of a well nourished girl baby, 73 cm. long. Rigor mortis was not present; postmortem lividity was slight. The pupils were equal. The lower middle incisors were present. The upper left middle incisor had just penetrated the gum. The body was still warm. There was nothing unusual in the peritoneal cavity except a moderate general hyperplasia of the lymphoid follicles. Apparently there was an increase in the number of visible hemolymph glands. The lower border of the liver extended 6 cm. below the tip of the ensiform and  $4\frac{1}{2}$  cm. below the costal margin in the right mammillary line. The peripheral lymph glands were not obviously enlarged. The thymus extended  $4\frac{1}{2}$  cm. below the sternal notch, was 4 cm. in width, and did not appear to be hyperplastic. It had two ascending limbs one of which passed forward anterior to, the other posterior to, the innominate vein.

When the sternum was removed, the lungs did not collapse. The lungs and heart were taken out *en masse* and as the trachea was cut across it was found to be practically filled with a thick yellowish mucoid purulent material.

The heart was of fair size but there was a tremendous dilatation of the right auricle, which was filled with a mass of deep red clot that was not laminated. The foramen ovale was open but seemed to be provided with a fairly competent valve. The tricuspid orifice admitted the tip of the little finger. The pulmonary orifice was tremendously contracted and permitted the passage of a probe not more than 3 mm. in diameter. The pulmonary artery itself was small. Immediately posterior to the pulmonary orifice was a very large orifice which led immediately into the left ventricle just below the aortic valves. The aorta was considerably dilated. The mitral orifice admitted the tip of the first finger. The aortic orifice on the left measured just 6 mm. in diameter and on the right, 1 cm. in diameter. There was evidently a defect in the interventricular sputum which permitted the pulmonary artery and aorta practically to join just above the muscular septum. The aortic leaflets seemed to be perfectly healthy, but the interventricular opening was not provided with any leaflets. The myocardium of the right side of the heart was somewhat thicker and more hypertrophic than that of the left side of the heart so that the right myocardium resembled more the usual musculature of the left heart. Both cavities were somewhat dilated, the left more than the right. The ductus Botalli was not evidently present, but in the usual position for it was what appeared to be an unusually large bronchial artery which led directly into the tissue of the right lung. This bronchial arterial branch led into a cavity, aneurismal in general appearance, just external to the right lung in the mediastinum, and was filled with a laminated clot.

The right lung was, as to the upper lobe and the upper two-thirds of the middle lobe, crepitant and healthy in appearance. The lower lobe and lower part of the middle lobe were filled with very numerous nodules in which there was no crepitation. On section, it appeared that these nodules were small foci of consolidation from which small, almost tubular casts of pus could be expressed. The left lung was, except for the presence of a few scattered patches of atelectasis, healthy.

The spleen was of fair size and measured 7x4x2 cm., was quite firm, a deep bluish-purple in color, and the Malpighian bodies were just visible.

The liver was large, quite congested, but showed nothing else unusual. There were no obvious lesions in the other organs. The kidneys, except for a moderate congestion and a certain amount of edema and some slight paleness, seemed to be healthy. The fetal lobulations were extremely distinct.

ANATOMIC DIAGNOSIS.—Congenital cardiac septal defect; patent foramen ovale; aneurism of an abnormal bronchial artery (right); acute lobular pneumonia.

#### REMARKS.

This very unusual case is interesting for other reasons than those associated with the aneurism. Nevertheless it is this that concerns us at this time. From the changes elsewhere in the pulmonary vascular tree it seems that there must have been some other factor at work than mere pressure,—that there must have been some toxic cause associated with the vascular anomaly. Throughout the consolidated portions of the lungs the vessels, branches of the pulmonary arterial system, were the seats of an extreme obliterating endarteritis, with which was associated thrombosis. The latter condition was most distinct in sections of the larger pulmonary arterial radicles in which these vessels are found to be almost completely filled with lamellated clots undergoing peripheral organization. In the smaller vessels there is evidence that many of them had been thrombosed and that the thrombi have been organized and canalized. In the smallest vessels the obliterating hyperplasia is clearest. It is probable that the agent which caused the clotting was also the cause of the weakening of the vessel that presented the aneurism. It is exceptional that this aneurism appeared in an anomalous bronchial artery.\* Reference may be made here to a case of Weller<sup>11</sup> in which there was an aneurism of the ductus arteriosus.

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\*For a general discussion of Congenital Heart Disease, see Hirschfelder, *Diseases of the Heart and Aorta*, 1913, p. 513, et seq.

# TOXICITY OF HETEROLOGOUS AND HOMOLOGOUS SERUMS

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IT has been known for a long time that normal blood and blood serum are, under certain conditions, poisonous substances, some much more so than others; but the nature of the toxic action was purely a matter of conjecture until a few years ago. Before attacking the question of the nature of this toxicity, it may not be out of place to give a brief outline of the development of ideas in connection with blood injection phenomena. These conceptions are, in some cases, not directly related to the subject in hand, but will serve to outline the steps by which our present attitude had been reached.

The first observation of this kind dates back to the year 1666, when an account<sup>1</sup> of a sudden death following blood transfusion was reported to the London Royal Philosophical Society, the cause assigned being that of intravascular coagulation.

The next important work of which there is any record is that of Hippolite Magnani,<sup>2</sup> who made use of sheep blood injections into dogs. He found that in many cases the animals died very suddenly, and concluded that death resulted from too large an injection. The same observer also gave the first description of hemoglobinuria associated with such conditions.

Bischoff<sup>3</sup> believed that the toxic effects were due to the fact that the blood used for injections had not previously been defibrinated. Later he reported that this made no particular difference, and assumed that death was caused by the venous character of the blood.

Magendie<sup>10</sup> (1839) reported extravasation and hemorrhage in the lungs and abdominal organs as a result of injection of defibrinated blood, thus presenting a definite anatomical picture for a condition hitherto an object of little more than speculation.

Panum<sup>4</sup> was the first to establish the fact that the foreign corpuscles function normally for a short time and are then destroyed and excreted in the feces and urine. Brown-Sequard claimed to have observed the foreign blood cells one month after injection.

Landois,<sup>5</sup> in a series of experiments with whole blood injections, concluded that death was caused by the combined action of two conditions:

1. Destruction of red blood cells and liberation of fibrin, thus leading to coagulation of blood and the formation of thrombi in the pulmonary capillaries.
2. Agglutination of red blood cells and the deposition of masses of agglutinated cells in the pulmonary circuit.

Neudorfer<sup>6</sup> ascribed death under such conditions to the presence of unsaturated fatty acids, his idea being that the damage was done to the organism through the saturation of such unsaturated compounds.

Ponick<sup>7</sup> injected defibrinated blood into the external jugular vein of a dog and observed respiratory symptoms coupled with other disturbances. He, however, discredited the hemagglutination theory of mechanical interference.

In 1877 Kohler<sup>19</sup> showed that fresh defibrinated blood, whether homologous

or heterologous, is an active poison, and ascribed toxicity to the presence of a fibrin ferment.

Moni<sup>8</sup> concluded that the toxic effect of the introduction of washed red blood cells was due to the hemolytic action of the serum of the recipient.

Batelli<sup>9</sup> held that serum must contain specific agglutinins in order to be toxic.

Gottlieb, Lefmann and Lefmann<sup>10</sup> studied the ether extractives of red blood cells and claimed to have demonstrated the toxic action of such substances on animals.

Coca<sup>10</sup> supported the contention that red blood cells contain certain substances, probably lipoids, which have a toxic effect when suddenly introduced into the blood stream of animals in large quantities. In summing up his own previous work he makes the following statements:

1. Cause of sudden death following injection of washed red blood cells rests in the clogging of the capillaries of the pulmonary circulation due to cell agglutination.

2. Presence of specific agglutinins in the serum of the animal is not in itself sufficient to explain the phenomenon. The additional fact of the interaction of a substance derived from the endothelial lining of the capillaries must be assumed.

3. Toxic substances in inactive form can be demonstrated in fresh corpuscles.

These ideas as to the mechanical nature of the toxic action of foreign blood and serum held sway in some quarters as late as 1910, for at that time three workers connected with the Rockefeller Foundation,—Loeb, Strickler, and Tuttle,<sup>11</sup>—brought out a paper in which they distinguished two kinds of serums; one, of which they said dog serum was an example, being hemolytic and coagulating, and the other,—beef serum—agglutinating. On the basis of these findings they joined the ranks of their predecessors and proclaimed the cause of death resulting from injection of heterologous serum as being twofold:

1. Stoppage of lung vessels with fibrin thrombi.

2. Stoppage of lung vessels with masses of agglutinated red blood cells.

During the past decade the study of serum toxicity has received great impetus through the discovery and development of immunity reactions and anaphylaxis. This was in part inevitable since serums played the principal role in such processes, and investigators were continually meeting with unexpected results from the use of primarily toxic serums. Besides this, the phenomena of anaphylaxis and serum toxicity were so closely related that it was necessary to investigate both in order to understand either. Careful research soon demonstrated that serum toxicity, whatever its real nature, was certainly not a mechanical process, as previous workers had supposed, and precipitation, agglutination, and hemolysis were assigned their true place—independent of toxicity.

Some such serums were found to have a much higher primary toxicity than others, so that a single injection of even very small quantities proved fatal in a very short time. Of such serums that of the eel was the most highly toxic, being one thousand times more active than the most anaphylatoxic substance yet produced. Human serum, as well as rat, beef, and goat serum belonged to this highly fatal class. Norry<sup>12</sup> finds that normal rat serum injected into a guinea pig gives a typical colloidal periodicity curve of toxicity, and that this toxicity

is very greatly increased by the addition of water. *Doer* and *Raubitschek*,<sup>13</sup> working in 1908, found that the purely toxic part of eel and beef serum was separate and distinct from the sensitizing and anaphylactic substances, and could be destroyed comparatively easily by heat and dilute acids. Thus, if the toxic portion was destroyed, it was found that the remainder was capable of producing all the phenomena of sensitization and anaphylactic shock. However, these observers, as well as *Kossel*,<sup>14</sup> showed that, as the primary toxicity of a serum increased, the hypersensitizing and anaphylactic properties seemed to decrease and were in some cases masked altogether.

Perhaps the most comprehensive work done in recent years on primarily toxic serums is that of *Zinsser*,<sup>15</sup> who utilized rabbits for making goat serum injections. He found that the severity of symptoms increased with the size of the dose and that the symptoms themselves were very similar to those described by *Uhlenhuth* and *Haendel*, as well as by *Doer* and *Moldavan* in connection with their work on anaphylaxis. He reported the following:

Latent period varying with the quantity injected from 1 to 10 min.

Respiratory distress.

Paralysis, beginning with hind legs.

General convulsions before death.

Following the work of *Doer* and *Raubitschek*, *Zinsser* investigated the nature and properties of the toxic portion of the serum he had used. He first tried to establish the relation of toxicity to associated characteristics of goat serum, such as agglutination and hemolysis, and to this end he utilized the previously demonstrated fact of the heat lability of the toxin. The serum was heated at 56° C. for twenty minutes, and it was found that both toxicity and hemolytic power had been destroyed, but that the agglutinating capacity remained. Reasoning from this result it was concluded that agglutination, at least, could not be regarded as the toxic factor, but the experiment had shown that the reaction of hemolytic and toxic portions were the same. Were they actually identical? In order to test this, *Zinsser* removed the hemolytic amboceptor and complement from the serum by allowing the latter to act on washed red blood cells until the hemolytic power was exhausted. The substance remaining was centrifuged to remove the cells, and injected into guinea pigs, with results that clearly indicated that toxicity was unimpaired. Hemolysis was, therefore, also ruled out as the toxic principle, and only two possible interpretations remained. The poison must be either an anaphylatoxin, produced by the action of the serum on the cells, or a heat sensitive toxin independent of hemolytic property. Further investigation established the latter as the more probable, for,

1. The hemolytic property of inactivated goat serum could be restored with guinea pig complement, whereas toxicity could not.

2. Toxicity dropped more than the hemolytic property on standing.

3. The toxic substance after removal of the hemolytic amboceptor and complement corresponded closely to the toxic dose of the original serum.

4. The toxicity of the fluid remaining after the removal of the hemolytic amboceptor and complement was destroyed at 56° C.

From these results *Zinsser* concluded that the toxic action of goat serum for rabbits was due to a heat sensitive substance independent of agglutination and hemolysis.

The question now arose as to whether the reaction might not be anaphylactic, but investigation soon showed that this was not the case, for, according to Zinsser, the reactions differed in the following particulars:

1. *Autopsy*.—No lung inflation; gross changes practically absent.
2. *Temperature Fall*.—"Although this does occur, it means but little, since in this case an active serum was used, distinguished from the inert protein matter used in such work."
3. *Diminution of Complement*.—Occurred in all cases in spite of the fact that the complement of the subject had been shown to have no part in the reaction. This was proportionally less than in true anaphylaxis.
4. *Antianaphylaxis*.—Rabbits which received sublethal doses showed no increasing resistance to subsequent injection. "It appears certain that nothing of the kind takes place."
5. *Fall of Blood Pressure*.—Preliminary rise followed by slight fall and rise to normal. In young rabbits fall was rapid and continued until death.
6. *Etherization* did not prevent death.
7. *Atropin* had no effect.

Doer and Raubitschek,<sup>16</sup> and Uhlenhuth and Haendel<sup>17</sup> had shown the same thing by heating the serum at 56° C. and finding that the toxic portion was destroyed while the anaphylactic portion remained.

Like other toxins those of normal serum produce antitoxins which protect against future injections. This process goes on simultaneously with that of sensitization in serums, such as the eel, which possess double antigens, so that it is possible, as shown by Doer and Raubitschek, to have an animal immunized to eel toxin and sensitized to eel anaphylactogen, with the result that on the second injection a double process occurs; namely, that of toxin binding and anaphylactic shock. This double phenomenon can further be demonstrated by transferring blood from an injected guinea pig to a normal animal, and thus establishing, on the one hand, passive immunity to the toxin, and on the other, passive sensitization to the anaphylactogen. It can, therefore, readily be seen why it is impossible to immunize an animal against such serums; immunity to the toxin can be established readily enough, but there can be no immunity to anaphylaxis, and it is the latter which produces the symptoms or kills on the second injection.

As to the actual nature of this toxicity, nothing absolutely definite is known. Early experiments with defibrinated blood had naturally enough suggested the idea that toxicity was associated with coagulation, and investigation seems to have confirmed this, as the following experiments by Novy<sup>18</sup> indicate.

If rabbit blood is taken and transferred directly to a guinea pig at high speed—30 secs. from heart of rabbit to vein of guinea pig—no toxic action is evident. However, if the animal is bled and the blood defibrinated, toxicity becomes evident, and furthermore, it is found that the degree of toxicity varies with the mode of defibrination. Thus, if defibrination is accomplished by shaking with glass beads the product is highly toxic, whereas defibrination with an iron wire yields a blood of mild action. Serum obtained by rapid centrifugation of defibrinated blood is also poisonous.

The relation of such toxicity to coagulation has brought forth a variety of theories. *Kohler* ascribed toxicity to the presence of the fibrin ferment; *Studzinski* held that the poison might come from mechanical disruption of the red

blood corpuscles, and *Freud* thought that the poison came from injury to the blood platelets. No one theory explaining the phenomenon has been accepted, at least each has found its opponents, so the whole matter must still be regarded as an open question. However, we may accept the statement of *Vaughan*<sup>19</sup> that "serums, even of homologous origin, behave like foreign proteins possibly on account of changes that take place during coagulation."

This *primarily* toxic property of serums, while of great interest and importance, is far outweighed in significance by another phenomenon associated with the poisonous action of serum, namely, that of hypersensitization or anaphylaxis. This phenomenon is not in the strictest sense a toxic action, since it has definitely been proved that no toxin—used in the sense of a substance which calls forth an antitoxin—is involved. However, considering our subject from its broader aspect, namely, toxicity in the general sense of poisoning, the question of serum anaphylaxis cannot be omitted. A brief outline of the development of this subject will perhaps serve to give a general viewpoint not otherwise obtainable.

As early as the year 1839, Magendie noticed that if an animal received an injection of a given substance, and later received a second injection of the same substance it usually died immediately, or at least after a very short time. However, neither he nor his contemporaries followed up this observation so that it was soon forgotten.

The early observations made by *Behring*<sup>20</sup> and his pupils (1893) in connection with complications arising from the use of antitoxin, as well as similar conditions noted by others with regard to tuberculin and mallein, are now regarded as purely anaphylactic phenomena, but at that time more or less labored explanations were formulated on the basis of Ehrlich's side chain theory of immunity. It was not until 1898 that the fundamental observations now at the basis of our knowledge of anaphylaxis were made by *Hericourt* and *Richet*<sup>21</sup> while working with eel serum injections into dogs. They observed that repeated injections gave an increased susceptibility to the serum instead of the expected immunity. Later *Portier* and *Richet*<sup>22</sup> made similar observations while working with "Actino-congestin," a poisonous substance derived from the tentacles of *Actinia*. This substance in minute doses produced severe symptoms and death in dogs. If a sublethal dose was given and a few days allowed to elapse before a second injection was administered, it was found that one-third to one-fifth of a lethal dose produced death. From these experiments it was concluded that the first dose had given rise to a well marked increase in susceptibility so that on the second injection a very greatly decreased quantity produced severe symptoms and death. *Richet* coined the word anaphylaxis (*ana*-against; *φύλαξις*-protection) to distinguish this phenomenon from immunity or prophylaxis. This term has since been shown to be a misnomer (*Vaughan*).<sup>23</sup>

Shortly after *Richet*'s earlier experiments, *Arthus*<sup>24</sup> made a series of observations in connection with the repeated injection of horse serum into rabbits and worked out a condition which has since been known as the "Arthus Phenomenon." He repeatedly injected horse serum subcutaneously at short intervals, and found that he was able to produce an intense local reaction which, with continued dosage, progressed from a mild edema at the site of injection to a



hard induration followed by subsequent sloughing off and ulcer formation. This showed that with each succeeding injection the susceptibility of the animal was raised until a point was reached where the same quantity of serum which had at first caused only slight edema was capable of producing such intense action that sloughing of tissue resulted. An observation very similar to that of Arthus was made by Theobald Smith<sup>25</sup> in 1904. He noticed that guinea pigs injected with mixtures of toxin and antitoxin in the course of antitoxin standardization died if after a short interval they were reinjected with normal horse serum. At about this time Otto<sup>26</sup> and Rosenau and Anderson<sup>27</sup> made elaborate inquiries into the nature of this reaction and succeeded in bringing out many new points, such as specificity of the reaction, transmission of hypersensitivity to the offspring, etc. "Die Serumkrankheit," a treatise by von Pirquet and Shick<sup>28</sup> also appeared at this time. The breadth of the biological principle involved was shown by Vaughan and Wheeler,<sup>29</sup> Nicolle,<sup>30</sup> and others who demonstrated that the reaction was not confined to animal serums but was elicited by proteins in general.

Needless to say a phenomenon of so striking a nature at once made its influence felt in other directions and the mechanical obstruction theory of serum toxicity began to lose ground. It now became an object to establish definitely the relationship of anaphylaxis to the associated phenomena of hemolysis and agglutination. The researches of a number of observers soon established this. Uhlenhuth and Haendel<sup>31</sup> found that inactive beef serum activated with guinea pig complement was able to hemolise guinea pig red blood cells in vitro, but had no anaphylactic action; they therefore said:

"The anaphylactic substances are probably not identical with hemolysins, hemolytic amboceptor or complement."

Doer and Moldovan<sup>32</sup> reported findings similar to the above but disagreed with some other observers who claimed to have destroyed hemolytic and agglutinating properties without injuring toxicity by heating at 50° to 60° C. Attempts to separate the hemolytic and toxic properties by either chemical or physical means—heat, alcohol, carbon dioxide, dialysis, etc.,—failed entirely, in fact a remarkably parallel reaction was evident. However they too believed that toxicity and hemolysis were separate, and summed the matter up by saying, "Toxicity is independent of hemolysis even though it is caused by the same amboceptor." The same authors "find it reasonable to suppose that toxicity is referable to an excessive precipitin reaction."

The latter statement is in direct line with the conclusion at which Friedberger<sup>33</sup> arrived, namely, that precipitation and toxicity are simply different phases of the same reaction. In other words no differentiation other than a purely quantitative one can be made between precipitation and toxic amboceptor. This idea will be more fully discussed with the theories of serum toxicity.

No one, of course, doubts any longer that the so-called secondary toxicity of serum is identical with anaphylactic shock. Doer and Moldovan, for instance, find that the symptoms following injection of normal serum are not only externally but from their very nature anaphylactic in type; guinea pigs show the typical Auer-Lewis phenomena and symptoms can be antagonized through the use of atropin.

The relationship of this type of serum toxicity to associated phenomena is well expressed by the following quotation from Vaughan's "Protein Split Products."

"It seems most probable that anaphylactogens, agglutinogens, precipitinogens and lysinogens are identical. In other words one group in the protein molecule causes the animal to develop a substance which, under certain conditions, may act as an agglutinin, precipitin or lysin. We are inclined to the belief that the same ferment may, under varied conditions, act as an agglutinin, precipitin, lysin, or it may cause a deeper cleavage of the protein molecule resulting in the liberation of the protein poison."

The principal symptoms accompanying so widely known a phenomenon are, of course, definitely known and the accounts of various workers are very much the same although differences do arise from the use of different experimental animals. The most evident symptoms as described by Vaughan<sup>34</sup>—less concisely by Auer and Lewis,<sup>35</sup> and Gay and Southard,<sup>36</sup> and Biedl and Kraus<sup>37</sup>—make their appearance in three stages. The following quotation from Vaughan's "Protein Split Products" is an accurate description of the principal facts available:

"The first stage is that of peripheral irritation. The animal is excited and evidently itches intensely as is shown by its attempts to scratch every part of its body that it can reach with its feet. The second stage is partial paralysis. The animal lies on its side with rapid shallow and difficult breathing. It is disinclined to move and when urged to do so shows more or less incoordination of movement and muscular weakness, with partial paralysis especially observable in the posterior extremities, which it drags. Rarely the animal dies in this stage. The third or convulsive stage, begins with throwing back the head at short intervals. The convulsions become more general, more frequent and violent, and the animal having reached this stage, usually dies in a convulsion or immediately following one. Expulsion of urine and feces is common in the convulsive stage."

While the above symptom complex is described in connection with animals treated with Vaughan's protein poison, it is also an exact picture of conditions arising from anaphylactic shock produced in the usual way.

Observers are not altogether agreed as to the autopsy findings in cases of anaphylactic death. Gay and Southard were the first to make a thorough study of this phase of the question, and they came to the following conclusions:

Tissues of animals (guinea pigs) examined during the anaphylactic phase, that is, during the period of incubation between the first and second injection, show no lesions. However, the toxic phase is marked by striking changes. Eighty-five per cent of the animals dying after the second injection, or being killed within twenty-four hours thereafter, show macroscopic hemorrhage in one or more organs. The stomach leads in frequency of involvement (55 per cent) and the lungs come next in order (40 per cent). Other localizations of hemorrhage are to be found in the brain spinal cord and peritoneum. Hemorrhage is associated with widespread fatty degeneration of capillary endothelial cells, heart muscle, peripheral nerves, voluntary muscle and gastric epithelium.

Rosenau and Anderson<sup>38</sup> found congestion and sometimes hemorrhage, but

they say that these lesions are not always apparent and are not specific. Furthermore, they were unable to demonstrate fatty degeneration.

Auer and Lewis studied the lung picture at autopsy and found the lungs greatly distended and filling the entire thoracic cavity. On opening the chest the lungs failed to collapse and even on section showed no decrease in distention. The alveoli and air cells were filled with air imprisoned by the tetanic contraction of the muscles of the bronchioles. Slight edema was also noted.

Vaughan, in using his protein poison, which he has demonstrated as being identical with the anaphylactic poison, finds that in the pure state parenteral injection of the poison causes no lesions of the kind described by Gay and Southard, but if an incompletely purified product is used, intense hemorrhagic peritonitis results.

The findings of Gay and Southard, as well as Auer and Lewis, were confirmed by Biedl and Kraus, and it is to the latter that we owe the most satisfactory demonstration of the mechanism of anaphylactic shock.

As might be expected, Gay and Southard, being the first to describe the lesions associated with anaphylactic shock, immediately concluded that these were the direct cause of death. On this basis they held that the respiratory symptoms were due to a lesion at the respiratory center, the same reasoning applying to the other symptoms as well.

Rosenau and Anderson, while differing with Gay and Southard in the matter of the gross lesions, agree that death must come from primary involvement of the respiratory center. To quote from their own report: "It is suggested that the essential lesion of serum anaphylaxis is localized in the respiratory center."

Auer and Lewis disagree with these views and offer as evidence the fact that typical anaphylactic death can be induced in guinea pigs even after section of the vagi and sympathetic, thus showing that involvement of the respiratory center of the medulla is an unnecessary assumption in explaining the phenomenon.

The adherents to the colloidal theory of the nature of anaphylactic phenomena, among whom may be mentioned Zangger,<sup>40</sup> Novy<sup>41</sup> and others hold that the mechanism involved is simply one of colloidal displacement in the body plasma, no special localized area being assumed.

As stated above, the interpretation of Biedl and Kraus is in some ways the simplest and most convenient. They find that in dogs fall of blood pressure is a characteristic and constant result of reinjection, and, furthermore, that the fall in pressure parallels the progress of the symptoms. Accompanying this drop there is an increase in heart rate; this leaves open only one interpretation—the fall in pressure is the result of great peripheral vasodilation. It follows, therefore, that anaphylactic shock must express itself at least partly through vasodilation. Experiments made by stimulating both central and peripheral nervous system failed to bring about a rise of pressure, and adrenalin, which ordinarily causes great vasoconstriction, proved of very little or no aid; as this drug is known to act on the peripheral nervous apparatus in the vessel walls, it was concluded that the vasodilation of anaphylactic shock was due to the paralysis of the peripheral vasomotor apparatus. This explains why stimulation of either the vasomotor nerves or vasomotor center failed to produce a rise in pressure. A further proof of the probability of this view is found in the fact that if

barium chloride is administered before the second injection the blood pressure fall is avoided, and this salt is known to act directly on the smooth muscles of the vessel walls causing vasoconstriction. Having established the fact that the fall of pressure is a constant phenomenon in connection with anaphylaxis the authors conclude that the other symptoms are easily explained on this basis. Fall in pressure results in brain anemia, and as a direct consequence of the latter various symptoms of convulsion, paralysis, etc., are called out. However, this admirably convenient and simple conception fails to take into account the Auer and Lewis experiment of the cut vagi and sympathetics referred to above.

We have now considered the development of ideas concerning the nature of the phenomenon of serum toxicity, its relation to associated phenomena, its external and postmortem manifestations, and the ideas concerning the mechanism of symptom production. All that now remains is to discuss the theories as to the basic causes underlying these things.

Any discussion of the cause of a phenomenon must take into account the conditions met with and the influence they are likely to have on the final result. Von Eisler<sup>39</sup> has worked with the effect of salts and nonelectrolytes on lytic poisons and in the report of his work has given a rather comprehensive conception of the conditions and media in and through which a poison must act if it is to produce an effect. He holds that the permeability of membranes is closely associated with the toxic action of all substances. Such membranes being colloidal they are influenced by widely different factors, among which may be mentioned friction and temperature and the closely related factor of diffusion rate. Besides these, surface tension and the production of electrical potential must be considered.

In biological reactions we deal with colloids, crystalloids and electrolytes which are not independent but to a very large extent interactive and interdependent, and these substances in turn react with the highly complex structure of the cell plasma. The interaction of all these things is conditioned by, and dependent upon, the state of the colloids making up the cell membrane. From this statement of von Eisler it can readily be seen that the poisonous reaction of a serum may be a highly complicated process, a fact which it is well to bear in mind in considering the theories which various workers have advanced to account for serum toxicity.

The various theories of anaphylactic reactions and secondary serum toxicity can be grouped in two main classes. On the one hand we find a group of men who maintain that all the facts can be explained on the basis of physical or colloidal changes, and on the other we find a group that supports the idea of true poison involvement.

In the first volume of the *Zeitschrift für Immunitätsforschung* there appears an article by Zangger<sup>40</sup> in which immunity reactions are discussed from the standpoint of physical-colloidal changes. The following is a brief statement of the essential points brought out:

The substances entering into immunity reaction are colloids, and for this reason such reactions take place, not between homogeneous systems, but between colloidal systems. Thus colloids and colloidal reactions form the basis of the following changes: (1) separation processes such as precipitation and

agglutination, (2) processes of the opposite order, namely, lysis, solution, dispersion and increasing affinity of colloids for the media. The colloidal theory tries to explain the immunity and related reactions on the basis of these phenomena.

First of all the writer holds that the chemical view of the nature of these reactions is not justified, because the chemistry of the process is absolutely unknown at the present time and, as he says, without this a chemical theory is untenable, furthermore, to his mind, the chemical explanation does not cover all the facts.

The fundamental points of the colloidal theory are as follows:

1. The speed of a reaction is conditioned by the rate of passage between colloidal phases.
2. Equilibrium depends upon the distribution of the different phases, and upon the state and character of the limiting membrane.
3. The extent of the phases and the limiting membranes are of importance.

These laws are not only fundamental to the theory under consideration, but to the whole subject of colloidal reactions as well. This parallel is, therefore, taken as an argument in favor of the theory, and, furthermore, the laws known to govern the behavior of immune bodies under varying conditions of concentration and temperature, as well as those having to do with the influence of electrolytes, light, shaking, etc., seem to bear out this contention, at least to a certain extent.

From synthetic experiments the author also finds confirmation of his views. For instance, he finds that two colloids which independently have identical actions, when placed together do not combine their actions in the manner of a summation. In fact, one may actually neutralize the other. The degree of summation or neutralization depends on the relative concentration of the two substances. Besides this he finds that the course of such reactions is identical with that of immunity processes. Perhaps the most important of his synthetic colloid experiments are those in which he is able to substitute prepared colloids for the active substances in immunity reactions. Thus he says, "Known colloidal substances reproduce in many instances the scale of immunity reactions. Lipoids, particularly cholestrin in suspension in water, have an antitoxic action for toxins of such diverse origin as tetano toxin, tetano lysin, poison of bees, snakes, etc. Furthermore, many serums complemented with soaps give reaction characteristic of normal serums—heat liability, etc."

The scope of this theory, as well as its essential character, is given in the following classification of processes which he regards as being of purely colloidal nature:

1. Changes in membrane permeability, such as thickening, hardening, death due to alteration in substances transmitted, permeability resulting from the above, local destruction, such as opsonin action and phagocytosis, diffuse solution, such as lysis, etc.
2. Disturbances of cohesion—local destruction, dissolution of membrane, such as opsonin action and phagocytosis; solution, lysis.

The colloidal theory then, with its assumption of change in the physical state of membranes and plasma through disturbance and displacement of col-

loids, embraces all the phenomena of precipitation, agglutination, opsonin action, phagocytosis and lysis. The work of Novy and his associates which treats the phenomenon of anaphylaxis from the same angle, will now be considered.

The first observations were made in connection with anaphylatoxins and for these experiments agar, produced each time with definite technic, was used, since it was found that if physical conditions and the time element were not carefully taken into account the resulting product gave varying and unsatisfactory results. This agar suspension was incubated with rat serum and injected into a guinea pig and it was found that 0.25 c.c. killed. However, if this same substance was injected into a rat to the amount of 15 c.c., no poisonous action could be demonstrated. This latter point is of importance from the standpoint of the theory.

There was some reason to believe that by the incubation a ferment had been produced which was responsible for the action. To test this the serum was centrifuged and the clear homogeneous liquid was again injected, with the same result. This showed that if a ferment was present it must be a liquid, not a substance in solution. However, if such a ferment were present, progressive action in poison production should be noted and might be indicated by a fermentation curve. Successive toxicity tests brought out the surprising fact that toxicity did not steadily increase, but appeared and disappeared at regular intervals, first rising to a maximum and then falling off again to zero, the oscillations covering a period of about thirty minutes. Such a curve corresponds exactly with what is known as the colloidal periodicity curve. In the case of normal rabbit serum injected into guinea pigs, the significant observation was made that the addition of water tremendously increased the toxic action.

Since it was possible to produce a poison from agar in vitro the attempt was made to call forth the production of a similar poison in vivo. For some time attempts in this direction failed, but here, too, results were finally obtained, it simply being a question of getting the agar in the proper state before use. This experiment demonstrated beyond a doubt that anaphylactic shock could be produced in animals which had never been sensitized. This same phenomenon was shown by Vaughan in connection with his protein poison and may perhaps have a bearing on the subject of the primary toxicity of serums.

Probably the most striking outcome of these experiments was the production of nonspecific anaphylactic shock. The immune serum of a spirochete rat was applied to the organisms in low dilution (1:10) and agglutination took place. Later however, the organisms became active again. Tests were made with high dilutions (1:5000) and it was found that the organisms instantly showed great agitation and underwent almost immediate lysis. It seemed not improbable that some such phenomenon might be concerned in anaphylaxis. To test this out, a sensitized and a control animal were each injected with 14 c.c. of distilled water and it was found that the sensitized animal died of acute anaphylactic shock, while the normal animal showed no ill effects. This, then, constituted nonspecific anaphylactic shock, a phenomenon hitherto unknown. To confirm this result, attempts were made to reproduce the reaction in vitro, and these met with immediate success, as it was shown that poison

was produced from serum and distilled water in a powerful manner and at tremendous speed provided the dilutions were right.

The theory advanced to explain these results is as follows: Colloidal substances are born to function within certain limits. If anything is done to displace them permanently beyond their normal limits death follows. Thus Novy holds that proteins may exist in the aggregate, in colloidal suspension or in dispersion, and if a protein normally functioning in colloidal suspension is, for any reason, driven beyond the limits where a colloidal state is possible and remains there for any length of time, death results. A rebound to the first position usually takes place, and if this occurs before the animal has suffocated, recovery takes place. In such instances, moreover, there is often a secondary rebound shown in a secondary anaphylactic shock of a mild nature following the severe but nonfatal symptoms of the first. Such reactions are probably all in the direction of dispersion, and sensitization consists in a partial colloidal displacement which on the second injection is rendered complete.

Anaphylatoxin, then, is not a poison, but a physical state according to this view, and "it is possible to use anything under the sun and kill animals simply by altering the arrangement of the serum."

While the above views are maintained by some of the present workers, the majority hold to the idea that specific poisonous substances are involved. This conception of the phenomenon was really the first to be advanced, and like all other theories has gone through a series of evolutionary changes as a result of the addition of new facts from time to time.

One of the first theories in this connection, long since discarded, was that of Hamburger and Moro who supposed that the first injection called forth the production of precipitins and these on the second injection formed precipitates which interfered with the pulmonary circulation. This theory is in the same class with those advanced in connection with defibrinated blood transfer and falls to the ground principally because the symptoms produced are not of the type that might be expected from the presence of capillary emboli and furthermore no such emboli can be demonstrated.

The theory of Gay and Southard is of a different nature and, while not generally credited, still has its supporters. These observers briefly state their views as follows:

"It is clear that sensitization of guinea pigs to horse serum is associated with the persistence of an unneutralized portion of the serum in the sensitized animal and that the intoxication is due to some condition of the body cells which renders them particularly susceptible to those portions of the serum which have already been eliminated from the first dose."

In other words, a remnant of the first injection, "anaphylactin," remains in the blood of the sensitized animal, undigested and unchanged. This substance, which may be transferred from animal to animal, producing passive anaphylaxis, brings about a condition of hyperreceptivity or hypersusceptibility of the body cells for the homologous protein and on the second injection the violent interaction of these two factors causes the symptoms and death.

Richet thought that a substance in protein matter, "congestin," when injected into an animal called forth the formation of "toxogenin" in the animal

body. The second injection, carrying with it a second quantity of congestin, reacted with the toxogenin and produced a poison "apotoxin," which, acting essentially as a nerve poison, produced anaphylaxis. This theory is a close approximation to the more completely worked out and thoroughly understood theory of Vaughan and Wheeler, to be presented later. Whereas this theory imagines hypothetical and entirely unknown substances, that of the authors<sup>42</sup> mentioned presents fairly definite chemical groups which have not only been isolated, at least partly, but concerning whose chemical reaction many facts are known.

Bresredka thought that serum protein matter contained two substances which are active in producing anaphylaxis. During the period of incubation a substance called "sensibilogen" calls forth the production of "sensibilisin" by the body which at the time of the second injection reacts with the other protein substance, "antisensibilisin," to form a poison which acts in the central nervous system.

In 1909, Friedberger<sup>43</sup> advanced his theory of sessile receptors. He thought that at the first injection precipitins were formed which, not being produced in large quantities, remained attached to the cells as sessile precipitins. On the second injection these sessile precipitins were thought to react with the foreign proteins and produce anaphylaxis. The primary assumption of this theory of course was that a relationship existed between precipitins and anaphylaxis, a supposition which he explained by saying that the use of a large first injection caused the production of a large number of precipitins, of which some, from their very number, were detached from the cells and functioned as free precipitins. These free precipitins, he thought, were the cause of precipitate formation, whereas the sessile precipitins caused anaphylaxis. This interesting theory has been abandoned, partly on the ground of work done by its originator.

Two years later the same observer<sup>44</sup> found that by digesting precipitates with normal serum he was able to produce a powerful poison which he called "anaphylatoxin," a piece of work very similar to that of Friedmann in connection with blood corpuscle digestion. This poisonous substance was at once thought to be the true poison of anaphylaxis, and it seemed as though the problem of hypersensitization and anaphylaxis had been definitely solved. It soon became evident, however, that Friedberger's discovery had been credited with too wide a significance, for it was learned from the work of Bresredka and others that anaphylatoxins could be produced just as well from, for instance, starch and agar as from precipitates. This, of course, necessitated the discard of this theory as an explanation of anaphylaxis.

Perhaps the best known and most widely credited theory of anaphylaxis at the present time is that of Vaughan and Wheeler,<sup>45</sup> advanced in 1907, and differing from the Friedberger hypothesis, which it preceded by two years, in that it is based on known facts capable of chemical verification. These observers had found that by cleaving the protein molecule with dilute alkali in absolute alcohol they were able to separate a poisonous and a nonpoisonous part from proteins of the most diverse origin. The poisonous part was, as far as could be determined, identical in every case, whereas the nonpoisonous parts were not the same but proved to be characteristic of the particular protein from which



they were derived. This led to the conception that the protein molecule was made up of a nucleus or "archon" which was identical in all proteins and to which the secondary groups characteristic of each protein were attached.

Experiments made with the substances thus isolated gave interesting results. It was found that the injection of the poisonous part was highly fatal to experimental animals and that it killed with identical symptoms in all cases. Sensitization with this substance proved impossible and immunity could not be established. The nonpoisonous part gave rise on injection to phenomena of a wholly different order—it sensitized and was specific to the protein from which it had been derived, that is, it sensitized only to the homologous protein.

This, then, constituted a separation of sensitizing and anaphylactic substances, and demonstrated that the two substances were not identical. This had been suggested by previous observations and later work has brought additional confirmation. Thus Bresredka showed that serum heated to 50° to 60°C. lost its toxic property, but retained its ability to sensitize up to a temperature of 120°C.

Gay and Adler<sup>46</sup> separated the sensitizing and toxic portions by fractional precipitation with  $(\text{NH}_4)_2\text{SO}_4$ . One-third saturation gave the euglobulin fraction which was highly sensitizing but atoxic in its effect on animals, while the product from the two-thirds saturation gave a product which was at once sensitizing and toxic.

Kraus and Volk<sup>47</sup> found that heating serum at 100°C. for ten minutes destroyed the thermolabile toxic substance so that the serum was no longer able to produce anaphylactic shock, but the same serum was still able to sensitize.

The next step in the development of the theory was, of course, the investigation of these poisonous and nonpoisonous substances. This work was carried out with great thoroughness and a large number of reactions were worked out for both substances. Chief among these were the protein color reactions, though the nonpoisonous part was shown to respond to the Molisch test for carbohydrates. These researches left little room for doubt that the essential sensitizing and anaphylactic substances of serums were protein in nature, and this conclusion was indirectly substantiated by the positive and negative findings of other workers in the field.

Doer and Russ<sup>48</sup> concluded as a result of their studies in anaphylaxis that the sensitizing and toxic portions were associated with the globulins,—albumins, albumoses, and peptones being totally inactive.

Pick and Youmanouchi<sup>49</sup> obtained inconclusive results with serum lipoids as anaphylactogens. They found that single injections of such lipoids gave no sensitization, either active or passive, to subsequent injections of normal serum but that by repeated injections of normal serum both types could be produced.

The negative result of Sleeswijk<sup>50</sup> in attempting to establish the serum lipoids as the poisonous group responsible for anaphylaxis further strengthens Vaughan's<sup>51</sup> view. He worked with alcohol extracts of serum and found, to quote from his own report, that "serum lipoids are indifferent for sensitive animals. That portion of the serum which is insoluble in alcohol is practically without toxic effect and acts as a vaccine."

That the sensitizing and toxic groups are not identical can no longer be

doubted, but contrary to the views of most others, Vaughan and Wheeler deny that a mixed protein is necessary to produce the phenomena of anaphylaxis. They hold that the sensitizing and toxic portions are simply different groups of the same molecule, not actually separate substances.

These facts are the basis of the theory which follows—a theory whose consideration has been reserved until the last because it has suffered the least from adverse criticism and covers most of the points which others either fail to include or explain indefinitely and from pure hypothesis.

"In experimental anaphylaxis the first injection introduces into the body a foreign protein. This must be digested and the body cells slowly elaborate a specific proteolytic ferment which slowly digests it. In doing this certain body cells acquire a new function. The protein of the first injection is slowly digested, usually without the development of recognizable effects. . . . After the protein of the first injection has been disposed of, the new ferment in the form of a zymogen continues to be formed in the cells and on the second injection, after the proper interval, this zymogen is activated and splits up the protein so promptly and so abundantly that the liberated poison induces the symptoms of anaphylactic shock."

It is wholly beyond the scope and aim of this paper to distinguish between the two great schools of anaphylactic theory whose development and essential conceptions have been herewith outlined. The question has been and still is open, and further investigation alone can determine which is correct and tenable in the last analysis.

#### SUMMARY.

Serum toxicity, in its broadest sense, involves two types of phenomena,—primary toxicity and serum anaphylaxis.

Primary toxicity is due to a heat sensitive toxin independent of hemolysins, precipitins, agglutinins and anaphylactins.

In anaphylaxis the substance called forth by the parenteral injection of the sensitizing dose may under varied conditions act as an agglutinin, precipitin, lysin, or anaphylactin since it is probable that the same element is concerned in all cases.

The symptoms of anaphylaxis are practically identical in every case for animals of the same species. The symptoms vary in different species.

Reports on autopsy findings in cases of anaphylactic death do not wholly agree.

The mechanism of anaphylactic symptom production is still a matter of theory.

Two schools of thought as regards the theory of anaphylaxis exist at the present time: the physical colloidal theory, and the theories of true anaphylactin involvement. Each has its strong claims so that the question of which is correct is still open.

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## CONCERNING THE CHEMISTRY OF PERNICIOUS ANEMIA

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**I**DIOPATHIC pernicious anemia is by some considered essentially a disease of the gastrointestinal tract, involving atrophy of the mucosa and absorption either of enterogenous poisons or protein split products. By others it is considered a disease due to the hemolytic action of toxins elaborated in disease processes, and still others believe hypersplenism to be the causal factor.

Cederburg<sup>1</sup> holds that theoretically, at least, we can assume that by constant parenteral splitting of proteins and the entrance of split products into the blood stream a condition can arise which leads to a severe pernicious anemia. He calls attention to the similarity of the clinical findings in pernicious anemia with those of anaphylaxis and poisoning by protein split products. Assuming a constitutional weakness of the alimentary canal (constitutional predisposition of Schauman) a slight destructive process of the intestinal mucosa would be sufficient to break down the barricade against foreign proteins. A small amount of slightly changed protein could then pass into the blood stream and sensitize the organism against various foodstuffs. Eventually the individual becomes sensitized to practically all protein foods, so that after each meal partly changed albumoses pass through the mucosa, and are quickly broken down with liberation of protein poison. These split products have been shown to be not only hemolytic, but to act as a strong stimulus to the hematopoietic organs (Schittenhelm, Weichard und Grissamer<sup>2</sup>). Furthermore, in protein poisoning or anaphylaxis, severe destruction of the intestinal mucosa has been observed by Schittenhelm and Weichard,<sup>3</sup> Richet,<sup>4</sup> Manwaring,<sup>5</sup> and Edmunds.<sup>6</sup> Having once become permeable, a vicious circle is instituted. The intestine permits increasingly large amounts of unbroken protein to leak through, with the result that there is an increasing liberation of protein poison and consequently greater hemolysis; marked stimulation of the bone marrow, and a pernicious anemia picture of progressively increasing severity. While Cederburg<sup>1</sup> regards the intestinal canal as perhaps the usual source of the protein products, he calls attention to the fact that in diseases of any kind where there is tissue and organ destruction an anemia can arise resembling in every respect the classic cryptogenic form. For example, the anemia of pregnancy is quite probably due to the liberation of protein split products. That protein poisons do play an active role in the disturbances accompanying pregnancy is an acknowledged fact. Eclampsia, for example, is undoubtedly due to anaphylaxis (Weichard<sup>7</sup>). The anemia of lues, of malaria, and of tuberculosis may all be attributed to the action of protein poisons liberated through tissue destruction. The presence of intestinal parasites such as uncinaria, tania saginata and especially bothriocephalus latus may, where there is already a predisposition to weakness of the mucosa, suffice to set the anemic process in motion. Another causal (auslösender) factor of importance is chronic inflammation of the intestine which very quickly causes a mucosa already constitutionally weak to become permeable to incompletely digested proteins. Protracted or chronic

anaphylaxis leads, Cederburg thinks, to an antigen-antibody-complement reaction, whereby a very different poison is set free. Or, if one uses the terminology of Vaughan, a ferment is elaborated which tears down the foreign protein or substrate and liberates the toxic element. The poison acts indirectly: first through the liver and secondarily through the intestine (Manwaring<sup>7</sup>) in producing anemia. It must be assumed that in the portal system parenteral protein digestion occurs, and protein poison is here produced which exerts its hemolytic action on the red cells.

The peculiar remission periods are homologous, Cederburg<sup>1</sup> thinks, with the anallergic phenomena of anaphylaxis. After severe anaphylactic shock (clinically comparable to extreme intoxication) an animal is refractory for a certain period against the specific protein. The anallergic condition comes on suddenly and manifests itself as a blood crisis. Erythrocyte destruction ceases together with the attendant overstimulation of the hematopoietic organs. Consequently the bone marrow begins to exert its normal activity and the rapid improvement in the blood picture is made clear. Vaughan<sup>8</sup> regards the antianaphylactic state as due simply to the development of tolerance. To enter into a discussion of the theories regarding antianaphylaxis would carry us far beyond the scope of our subject. Whatever the actual mechanism of the process may be, the anallergic state passes once more into that of allergy and again the protein poison exerts its deleterious action. This cycle of remission and recidive repeats itself until the hematopoietic system is exhausted.

In connection with the subject of protein split products, some of the phenomena seen in pernicious anemia of horses are worthy of note. From the stomachs of horses suffering with the disease, larvæ of a fly (*Cæstridæ*) have been obtained from whose bodies an exceedingly active thermostabile toxin, œstrin, has been isolated. Injection of œstrin into the blood of healthy horses causes an anemia resembling in all respects the typical horse pernicious anemia. If blood plasma from horses sick with the disease is injected into healthy ones, the infection is transmitted and pernicious anemia results. In this latter case, however, the poison element is thermolabile, and heating for one hour at 58° destroys its virulence. Seyderhelm<sup>9, 10, 11, 12</sup> found the so-called virus in the blood serum, urine and feces, but not in the sputum. Subcutaneous or intravenous injection of serum filtered through a Chamberland or Berkefeld filter and which yielded negative results upon microscopic and bacteriologic examination, when injected produced in every case the typical anemia, provided the subject was a horse or mule. Injections into other species of animals failed. It is certainly very plausible, theoretically, to regard the virus of Seyderhelm as serum containing a specific proteolytic ferment capable of liberating protein poison. Injection of the ferment containing serum passively sensitizes the horse against some protein which we must assume is one of the constituents of its own body since the phenomenon occurs only upon injection into a horse or mule. Vaughan<sup>8</sup> has shown that serum and organ extracts from sensitized animals are inactivated when heated to 56° for thirty minutes, clearly harmonious with the thermolability of Seyderhelm's virus. Moffitt<sup>13</sup> has injected into horses human blood from cases of pernicious anemia with uniformly nega-

tive results as would be expected on the assumption of specific ferments as a causal agent. Mathes' assistant, Hunter, injected human blood from a pernicious anemic patient into a monkey and also obtained negative results (Mathes<sup>65</sup>). If injected into an anthropoid ape or into man one might expect symptoms of pernicious anemia to result, but in a horse or monkey the negative results could be predicted.

#### HEMOLYTIC TOXINS AS THE CAUSE OF PERNICIOUS ANEMIA.

That pernicious anemia is the result of a hemolytic toxemia is a theory which has received much consideration. A large number of chemical substances such as cestrin, oleic acid, saponin, pyrocin, acetanilid, phenylhydrazin, nitrobenzol,  $\beta$ -imino-azolyethylbenzaldehyde, toluylendiamin, and p-oxyphenylethylamin are active hemolytic agents and injection of suitable doses will produce hemolysis with attendant hemoglobinuria and anemia. In connection with pernicious anemia those hemolytic poisons which arise during the course of digestion or by putrefaction in the intestine are of especial interest. Iwao<sup>14</sup> has been able to isolate p-oxyphenylethylamin from autolyzing pancreas, putrefying horse flesh, and Swiss cheese. It is quite evident that this base may normally arise in the intestine from putrefying food, and in fact Berthelot and Bertrand<sup>15</sup> have isolated from human intestinal contents an organism of the Friedländer pneumobacillus group which they have named *B. aminophilus*, and which is able to form p-oxyphenylethylamin from tyrosin. In fact even the colon bacillus may, under certain conditions, produce this base from tyrosin. Iwao<sup>14</sup> concludes that among the toxins of intestinal origin p-oxyphenylethylamin is one capable of markedly changing the blood properties, and which experimentally can produce in animals an anemia with a picture similar to that of pernicious anemia. Barger and Dale<sup>16</sup> have isolated another hemolytic amin,  $\beta$ -imino-azolyethylamin from the mucosa of the small intestine of an ox. This substance when injected causes hemolysis and a general picture of anaphylactic poisoning with the exception that there is probably no change in the clotting time of the blood. Biedl and Kraus<sup>17</sup> hold, however, that this poison does delay clotting. Mellanby and Twort<sup>18</sup> have shown that  $\beta$ -imino-azolyethylamin can be formed from histidin in the intestine by the action of a bacillus which will grow only in alkaline media. In absorption of such toxic amins is to be found a very plausible explanation for the origin of anemias. Grawitz,<sup>19</sup> on the same basis, held coproastasis an important factor in pernicious anemia, and believed that through reabsorption of toxic substances arising from increased protein decay a general destruction of erythrocytes may occur with a resulting progressive anemia. In many cases atrophy of the intestinal mucosa is present, and to a vicious circle resulting from the decreased digestive and assimilative power Grawitz attributes the progressive character of the disease. He suggests also the possibility of fetal toxins being absorbed in pregnancy and leading to the anemia sometimes seen in such cases. On the basis of experimental investigations in which he found no increase in either the soluble or fatty acids or ptomaines in the urine of patients sick with pernicious anemia, Bloch<sup>20</sup> contradicts Grawitz's theory regarding the toxic origin. Berger and Tsuchiya<sup>21</sup> investigated extracts obtained from the mucosa of animals sick with anemia

apparently identical with the idiopathic pernicious form, and they found such extracts to have a much stronger hemolytic power than extracts from the mucosa of normal animals. Ewald and Friedberger,<sup>22</sup> on the contrary, assert that extracts of the mucosa of such animals are no more hemolytic than normal.

Faust and Tallquist<sup>23</sup> demonstrated that in bothriocephalus infection anemia is due to oleic acid which in the form of cholesterol oleate is found in the proglottis of the parasite. In the host this salt is split and the free oleic acid liberated combines with alkalis of the blood and is absorbed as soap. Thus the acid enters the circulation where it exerts its harmful action on the erythrocytes while the cholesterol radicle passes out unchanged in the feces. Tallquist<sup>24</sup> points out that the hemolytic action appears only upon dissolution of the worm segments and concludes that possibly the active hemolysin is enclosed, so to speak, in an albumin covering which must be broken down before the toxin can be freed and reabsorbed. Why the parasite goes into solution in some cases and in others remains intact is a phenomenon which needs to be explained, and an explanation of this would at the same time make clear why some cases of bothriocephalus infection lead to anemia while others do not.

If pernicious anemia is produced by hemolytic toxins, it is not unnatural to expect that highly active hemolysins should be found in the bodies of patients dying from the disease. McPhedran<sup>25</sup> prepared extracts by mincing organs in alcohol and so treating the filtrate that in the end he obtained two fractions, the first containing in addition to simple glycerids and free fatty acids cholesterol and its esters if they were present, while the second fraction contained only acetone insoluble substances. In the majority of cases cholesterol and unsaponified matter was removed from the first fraction before testing. Upon comparing the hemolytic power of such organ extracts from cases of cancer of the prostate, pneumonia, secondary anemia, and idiopathic pernicious anemia, he found that the figures obtained in pernicious anemia fell entirely within the limits of those obtained in other conditions and gave evidence of the presence of none of the hemolytic toxins supposed to be present as the cause. From the liver in cases of acute yellow atrophy and phosphorous poisoning Joannovics and Pick<sup>26</sup> obtained fatty acids which were not only very active hemolysins, but could actually be demonstrated as present in the blood. They suggested, therefore, that the hemolytic effects of such liver extracts were due to the fatty acids produced. By the tissue destruction of the toxin these unsaturated fatty acids of the liver are freed from their normal lecithin-like combination and are in a sense activated. In toxic anemias in general these investigators<sup>27</sup> looked for the cause of the hemolysis in the fatty acids liberated from cells destroyed by the action of the poisonous principle. The toxins causing pernicious anemia, if toxins are the cause, may act in this same manner.

Closely associated with the view that the hemolytic activity of pernicious anemia is due to fatty acids is the theory that a general disturbance of the lipid mechanism of the body is the primary cause of the disease. Chemical examination of the cortex of the adrenals in such cases shows it to be filled with lipid material and especially cholesterol and its esters. It is quite probable that this tissue exerts an influence on metabolism through its cholesterol control. (Howell<sup>28</sup>) Two

cases are cited by Kinnicutt<sup>29</sup> both of which at autopsy showed lipoidosis of the suprarenal cortex. In one case the degeneration was so complete that in places only fat infiltrated stroma remained. Hueck<sup>30</sup> has found that in pernicious anemia the lipoid content of the suprarenal cortex is increased. Landau<sup>31</sup> likewise found that there was a marked disturbance in the cortical tissue, and he thinks this of considerable significance because of the intimate relation which the cortex bears to the control of the cholesterol content of the blood.

The role played by the spleen in hemolytic processes is more or less uncertain, and the various views regarding its chemistry will be discussed in detail later. Moffitt<sup>32</sup> considers that a hypersplenic condition is brought about by toxins which reach the spleen through the lienal artery. Erythrolysis does not take place in the spleen, but in some way the erythrocytes are sensitized and prepared for later destruction in the liver, marrow, or lymph glands. Weidenreich noted that in pernicious anemia the lumen of the central artery of the spleen is so obliterated as to hinder the normal blood flow and force an unusually large amount of blood to travel by way of the capillaries directly into the pulp area. Once in contact with the connective tissue spaces of the pulp area, the erythrocyte is sensitized and marked for destruction. No attempt is made to explain this sensitization from its chemical or mechanical standpoint.

#### CHEMICAL CHANGES IN THE BLOOD.

In practically every case of pernicious anemia there is marked delay in the clotting time of the blood. This fact has been already mentioned in discussing the possible relation of pernicious anemia to anaphylactic phenomena. Drinker and Hurwitz<sup>33</sup> made a careful study of the factors of coagulation in pernicious anemia and determined quantitatively the different elements concerned in the phenomenon. They concluded from their investigations that prothrombin is diminished slightly in all cases, but that this diminution is not great and is unimportant provided active regeneration is in progress. Antithrombin and fibrinogen were found to be normal, even in the presence of a very low cell count. In one case in which there was pronounced diminution of prothrombin, the platelet counts were strikingly low. Hurwitz<sup>34</sup> has been able to show that fluctuations in the amount of prothrombin may be produced by substances affecting the number of platelets, and any toxin which produces a reduction in their number will simultaneously diminish the prothrombin of the circulating blood. No definite parallelism, however, has been established between bone marrow destruction, the number of blood platelets, and the amount of prothrombin. This suggests, therefore, that a reduction in the number of blood platelets alone will not cause a dangerous prothrombin deficiency; some other tissue must play a part in its formation. In his experimental aplastic anemias Hurwitz<sup>34</sup> found that the antithrombin and fibrinogen fluctuated but little from normal. Minot and Denny<sup>35</sup> agree in general with Hurwitz and Drinker's work, but found in two of their cases of pernicious anemia an increase rather than diminution in the prothrombin content. Furthermore, they found an abnormal antithrombin content while Hurwitz and Drinker did not. Moffitt<sup>32</sup> agrees with the authors cited regarding the diminution of blood platelets (and prothrombin) in pernicious anemia, and points out that splenectomy may be followed by a great increase in their number. Regarding the calcium



content of the blood, nothing is said, nor is mention made of any change in the viscosity.

The possible presence of specific proteolytic enzymes in the blood of individuals sick with pernicious anemia has already been mentioned in discussing anaphylactic phenomena as a hypothetical cause of the disease. The very evident indication of the existence of such enzymes in the blood of horses with pernicious anemia is especially worthy of note. No successful attempts, however, have been made thus far to demonstrate conclusively that similar ferments are present in human blood.

Ruttan and Adami<sup>36</sup> analyzed blood serum obtained at an autopsy performed five hours after death. They found the specific gravity to be 1.0261 which is below the figures cited by them as normal (1.027 to 1.030). Proteins made up 5.2 per cent by weight of the total substance, and of these 2.3 per cent represented the globulin, and the remaining 2.9 per cent, the serum albumin fractions. The ash was found to be 0.875 per cent or about 12½ per cent above the figure usually given. The total protein as found by these workers was accordingly about 40 per cent below normal while in addition the ratio of the globulins to the serum albumins was changed.

The fragility of the reds in pernicious anemia is altered through chemical changes in the cell envelope. McNeil<sup>37</sup> found that in varied groups of anemias the resistance of the reds to saponin hemolysis was diminished in all, and was most diminished in severe pernicious anemia. In chronic or less severe pernicious anemia the resistance is no more lessened than in cases of severe secondary anemia, and even in the worst cases of pernicious anemia the diminution is less than that which occurs in hemolytic jaundice. Saponin hemolysis is considered by McNeil<sup>37</sup> as a test of the cholesterol value of the cell envelope. Being anti-hemolytic the cholesterol content is an important factor in anemic conditions and its lack in pernicious anemia is thought by this worker to be a permanent and progressive defect.

In relation to fatty acids in the blood, one has to consider first whether in hemolytic diseases such as pernicious anemia fatty acids enter the blood in increased amount; and second, whether the amount of fatty acid entering the blood parallels the severity of the disease. King<sup>38</sup> found that in pernicious anemia there was an increase in the total fats of the blood, a decrease in the free cholesterol, and a strikingly high iodine number. His values for these constituents are as follows: Total fats, normal average, 5.600; pernicious anemia, 8.376; free cholesterol, normal average, 0.811; pernicious anemia, 0.342; iodine number, normal average, 90.00; pernicious anemia, 225.00. He concludes, therefore, that there is a definite parallelism between unsaturated fatty acids in the blood and hemolysis. This view is more or less opposed to the work of McPhedran<sup>25</sup> on the relation of fatty acids to pernicious anemia. McPhedran tested the hemolytic power of various fatty acids and found the lytic dose for ½ c.c. of normal washed red corpuscles to be as follows:

Sod. oleate,	.027 to .030 mg.
Sod. palmitate,	.40 mg.
Sod. linoleate,	.037 mg.
Sod. erucate,	.066 mg.

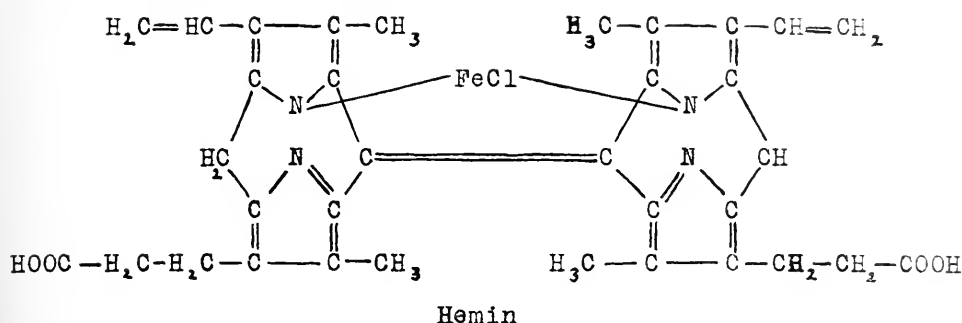
From these results it would appear that unsaturated fats with two or more double linked carbon atoms, as, for example, sodium linoleate are unable to cause any stronger hemolytic action than the simple double linked oleates, and McPhedran concludes, therefore, that hemolysis by fatty acids is not in proportion to the degree of unsaturation. In the blood of pernicious anemia patients the increase in total fats as shown by King was about 49 per cent while the increase in the iodine number was 127 per cent, indicating almost three times as high a degree of unsaturation. If this increase, as McPhedran believes, does not increase the hemolytic power, we must look to the decrease in free cholesterin for its cause. Lamar,<sup>39</sup> however, found that linoleic and lino-*lenic* acids in the form of Na salts were able to dissolve pneumococci more rapidly than did sodium oleate. Moreover, he found that the intensity of lytic action on the cocci varied directly with the degree of unsaturation, and accordingly he reached the conclusion that the hemolytic and bacteriolytic activity of the soaps of unsaturated fatty acids is probably due in part to their avidity for protein and not wholly to their lipolytic ability. Eppinger<sup>40</sup> likewise notes that in conditions associated with hemolysis, such as pernicious anemia and hemolytic icterus, there is an increase in the unsaturated fatty acids in the blood. The weight of evidence, therefore, indicates that the fatty acids are increased in the blood in pernicious anemia, but whether or not the increase parallels the severity of the anemia is still unclear.

#### HEMOLYSIS IN PERNICIOUS ANEMIA.

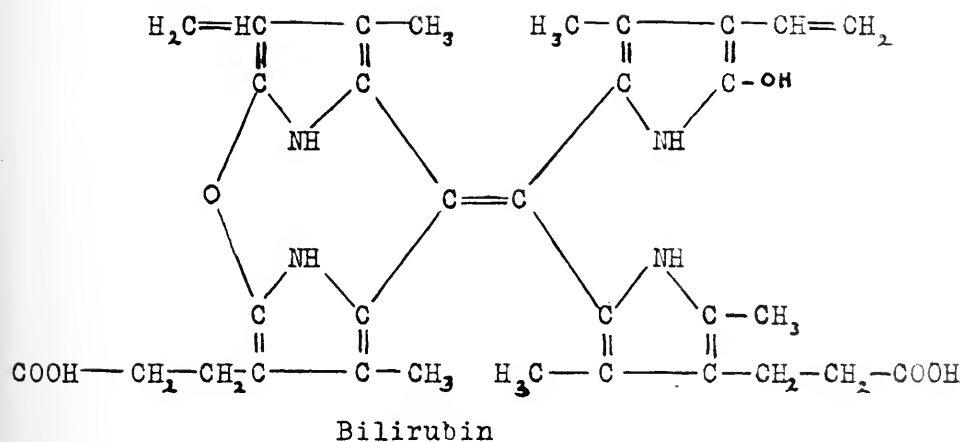
The hemolytic influence of foreign proteins, various poisons, fatty acids and lack of cholesterin has already been discussed. Here I wish to take up the question of the rate of destruction of erythrocytes as compared with the rate of regeneration, and the method of disposal of the products of hemolysis. The blood picture in pernicious anemia does not express the active processes going on, but merely indicates the mean,—the sum total of blood destroying factors set against the blood building factors. When the destructive factors are abnormally active, the hematopoietic organs are stimulated as has been already mentioned, and an attempt is made to counteract the loss. The actual amount of blood destruction is best determined by estimation of the secreted blood pigment. Schneider<sup>41</sup> believes that in the liver-spleen circulation the erythrocyte is disabled, that by contact with the spleen pulp it is in some way rendered more susceptible to hemolysis. Addis<sup>42</sup> considers that the influence of the spleen has something to do with cholesterin metabolism and attributes the change to activity of the peculiar endothelial phagocytic cells situated especially in the sinuses of the spleen, and to a less extent in the capillaries of lymph glands and bone marrow. The same type of cells stretching across the lumen of liver capillaries form the Kupffer or stellate cells of the liver.

The first step in hemoglobin pigment decomposition is the liberation of hemoglobin from the worn-out or damaged erythrocyte. Addis<sup>42</sup> remarks that the special function of the Kupffer cells is not only to phagocytose the red blood cells but to take up extruded hemoglobin as well, and droplets of hemoglobin have been seen by him passing from the Kupffer cells into the cells of the liver. In the liver cells the second step takes place and the hemochromogen of hemo-

globin is separated from the globin fraction. Outside the body this separation can be effected by treating hemoglobin with hydrochloric acid, hemin being formed. The structural formula as given by Addis<sup>12</sup> for hemin is as follows:



The mechanism of the subsequent process is not yet understood, but in some way the hemoglobin pigment is transformed by the liver cells into bilirubin. The extent of change can be seen by comparing the formula for hemin with that for bilirubin:



It will be noticed that iron has gone out from the molecule and the oxygen content is increased. That hemoglobin pigment does give rise to bilirubin Addis demonstrated by injecting hemoglobin pigment into the blood stream. An almost quantitative increase in the bilirubin excreted in the bile followed. For our purpose it will suffice to briefly sketch the subsequent steps in hemoglobin metabolism. After entering the intestine, bilirubin is reduced to urobilinogen. This is assumed to be absorbed in part from the intestine and polymerized into a hypothetical urobilin complex which is normally removed by the liver. The surest indication of generalized disturbance of the liver function is based according to Addis on a determination of the extent of failure of synthesis of hemoglobin pigment from this urobilin complex. Where active erythrocyte destruction is going on, it is natural to expect that the limit must be approached above which the liver can no longer dispose of the liberated pigment. That such actually is the case has been shown by Sellards and Minot.<sup>13</sup> These workers

found that compared with normal, less hemoglobin is required to produce hemoglobinuria in patients in whom there is increased blood destruction as, e.g., in pernicious anemia. In secondary anemia there is, as a rule, no excessive blood destruction and the hemoglobin necessary to produce hemoglobinuria in these cases approached normal. The amount of hemoglobin necessary to cause hemoglobinuria bears no relation to the red count, but is in direct proportion to the amount of blood destruction taking place. This last, providing complicating factors are absent, is directly proportional to the excretion of urobilin in the intestine. Eppinger<sup>40</sup> found that the normal urobilinogen excretion was 0.12 to 0.15 grams per day, while in pernicious anemia the excretion was 0.24 to 1.14 grams per day. Schneider<sup>41</sup> estimated the amount of hemolysis by examination of duodenal contents and found in pernicious anemia excessive excretion of bile pigments or pleochromie, and urobilinocholia. Pleochromie is an expression of immediate hemolysis and in pernicious anemia whether in crisis or remission is a constant finding. Urobilinocholia indicates a heaping up of pigment in the portal system and varies directly as the portal system is surcharged or becomes relatively empty of the plus of pigment. Regardless of whether or not gross liver changes can be demonstrated, urobilinocholia is highest in crisis. Schneider<sup>41</sup> points out that anemia caused by gastrointestinal hemorrhage, and in which the blood picture simulates that of pernicious anemia will definitely show an absence of pleochromia and urobilinocholia upon duodenal estimation of the bile pigments.

#### THE METABOLISM IN PERNICIOUS ANEMIA.

To Meyer and DuBois<sup>44</sup> is due the most valuable contribution on the basal metabolism in pernicious anemia, and their results will here be briefly reviewed. Five cases were studied in all of which there was an increased metabolism varying from 2 per cent to 33 per cent above the normal average. There are no profound qualitative metabolic changes in pernicious anemia as shown by the relationship between the  $\text{CO}_2$  produced and the  $\text{O}_2$  consumed, and fats and carbohydrates are burned to the same end products as in health. Clearly then, a reduction of hemoglobin does not preclude the possibility of either a normal or even an augmented metabolism. Normally the  $\text{O}_2$  supply to the tissues is beyond the immediate requirements, and in anemia, since there is no means of augmenting the  $\text{O}_2$  carrying capacity per unit of blood, the margin of safety from asphyxiation must be encroached upon with the result that the blood returns to the heart in various degrees of asphyxia. Plesch<sup>45</sup> has called attention to the fact that in anemia there is an increased heart rate. This suffices in uncomplicated cases to compensate for the increased demands per unit of blood in spite of the greatly diminished hemoglobin content. Morawitz<sup>46</sup> accounts for the increased  $\text{O}_2$  consumption and  $\text{CO}_2$  production in pernicious anemia by the presence of the nucleated reds whose  $\text{O}_2$  needs are extremely high. Meyer and DuBois<sup>44</sup> conclude that there are some grounds for the belief that the height of metabolism is a measure of the severity of the clinical picture. Peabody and Wentworth<sup>47</sup> found no change in the vital capacity of the lungs in pernicious anemia.

## IRON METABOLISM IN PERNICIOUS ANEMIA.

The importance of iron as a constituent of hemoglobin makes it a factor of much interest in the study of anemias. Whether an iron lack plays any role in pernicious anemia, and if so whether the feeding of preparations containing iron will be of any therapeutic value have been widely discussed questions. In chlorosis the therapeutic value of iron has long been acknowledged, but Morawitz<sup>46</sup> believes its value here to lie not in its effect upon the blood forming organs, but rather upon some other focus of disturbance, leading only secondarily to an increase in hemoglobin. Müller<sup>48</sup> concluded that inorganic iron salts added to an iron free diet in anemic animals increased erythropoiesis. Tartakowsky<sup>49</sup> held the same view. Abderhalden<sup>50</sup> also pointed out that inorganic iron as well as hemoglobin or hema-tin added to a diet poor in iron increased hemoglobin formation, but to a less extent than occurred when a diet of iron rich food was given. He concludes that whatever be the form in which iron is administered, it is absorbed by the same channels, stored in the same organs, and eliminated by the same routes, and he furthermore suggests that in all probability all iron assimilated, whatever its original source, is taken from the digestive tract in an ionized state. Hauserman<sup>51</sup> fed anemic animals on a low iron diet with  $\text{FeCl}_2$  added, another group he fed on a low iron diet only, while a third group received food poor in iron but with hemoglobin added, and a fourth group were fed a mixed diet of iron-containing foods. His results showed that  $\text{FeCl}_2$  was not an available source for iron to be changed to hemoglobin. Asher and Grossenbacher<sup>52</sup> found that after splenectomy in dogs there was a marked increase in the iron elimination. They concluded, therefore, that the spleen is an important organ in intermediary iron metabolism enabling the body to conserve and neutralize its supply. Austin and Pearce<sup>53</sup> obtained conflicting results and decided that there was no good evidence to support the view that the spleen exercised any fundamental influence on iron metabolism. Muir and Dunn<sup>54</sup> investigated iron retention in the organs in cases of hemolytic anemia using chiefly in their experiments guinea pigs made anemic by repeated injections of antiserum from a goat sensitized against guinea pig's corpuscles. The normal iron content of the organs in a 200 gram pig was as follows:

Liver,	4.64 mg.
Kidney,	0.54 mg.
Spleen,	0.24 mg.
Total,	5.46 mg.

In anemic animals the average percentage of iron in the liver and kidneys was about five times normal, and in the spleen it was about three times normal. In 200 gram severely anemic pigs the iron content was—

Liver,	25.02 mg.
Kidney,	3.69 mg.
Spleen,	2.49 mg.

Unless hemoglobin is present in the urine practically no increase of iron can be demonstrated in the kidneys, and the amount of hemoglobin escaping in the urine is roughly proportional to the amount of iron deposited. Muir and Dunn<sup>54</sup> emphasize the fact that a broad distinction can be made between the hemosiderin

deposits resulting from lysis and those resulting from phagocytosis of reds. In the former the iron deposit first appears in the cells of the peripheral zone of the liver lobule, and from thence spreads inward, giving at first a diffuse blue reaction, but later, beginning first at the periphery, granules of hemosiderin occur. In phagocytosis, on the contrary, the hemosideric deposits bear no special relation to the periphery, but occur rather throughout the liver lobules.

#### CHEMISTRY OF THE SPLEEN IN PERNICIOUS ANEMIA.

The spleen is supposed to exert a marked influence on iron metabolism. The increase in iron elimination seen by Asher and Grossenbacher<sup>52</sup> after splenectomy has already been mentioned, but is rather hard to understand. Certainly most workers have obtained contrary results. Pepper and Austin<sup>55</sup> found that in pernicious anemia the iron output through the feces, although never above normal, showed a decrease of 40 per cent after operation; while before splenectomy the excretion of urobilinogen and urobilin was about three times normal, the output two months after splenectomy was reduced to one-seventh of that before the operation. With reference to nitrogen they found that a slight positive balance before splenectomy was followed by retention fourteen days postoperative with a return to the preoperative balance after one month. The uric acid output showed a decrease of 22 per cent after the operation although it never exceeded normal limits. That the spleen is an important factor in blood destruction is evident from the decreased urobilin output following splenectomy. Pibram<sup>56</sup> points out that stasis of blood in the spleen, e. g., in chronic passive congestion, is associated with increased erythrolysis, and that urobilinuria can be induced by splenic congestion. Furthermore the blood crisis after splenectomy indicates either that the bone marrow has been stimulated to greater activity or that some inhibitory factor has been removed. The theory that bone marrow stimulation has occurred is supported by the increased number of platelets seen after splenectomy. On the other hand Klemperer believes that the spleen elaborates an inhibiting hormone for bone marrow. However, experiments made by Krumbhaar<sup>57</sup> demonstrated that fresh splenic extracts stimulated rather than inhibited the bone marrow, and furthermore Pearce and Pepper<sup>58</sup> have pointed out that the bone marrow activity does not begin until several months have elapsed after splenectomy. Krumbhaar<sup>59</sup> has indicated that it is difficult to prove that the lessened hemolysis after splenectomy is due to the absence of the spleen for neither normal spleens nor those removed in operation can be shown to possess any increased hemolytic activity. Furthermore analyses of blood entering and leaving the spleen have failed to throw any light on this subject. (Krumbhaar and Musser.<sup>57</sup>) After splenectomy the hemolytic resistance of the reds is increased, and this may be one of the factors leading to improvement after operation. Pearce and Karsner<sup>60</sup> found that splenectomy in normal animals was followed by an increased resistance of erythrocytes. Pearce, Austin and Evans<sup>6</sup> also found the resistance increased, while Joannovics and Pick<sup>62</sup> have shown in experimental animals that if the spleen is removed a much larger dose than normal of toluylenediamine is required to produce anemia. Joannovics<sup>63</sup> made fistula between the splenic vein and the left renal vein and in this way diverted the splenic blood into the inferior vena cava. Animals so treated were able to

tolerate toluylenediamine much better than could normal animals. He concluded that normally toluylenediamine to a slight extent but the spleen to a very much greater extent plays the active role in blood destruction. The decomposition products from the erythrocytes are, according to Joannovics,<sup>63</sup> taken out by the spleen and sent on to the liver through the portal vein. In this way an overabundance of material for the manufacture of bile pigment is at hand, and as a result the liver produces an excessive amount of concentrated bile. This leads to stasis, reabsorption and icterus. Krumbhaar<sup>59</sup> points out that whatever may be the cause for the improvement after splenectomy, the subsequent relapse is probably due to other organs such as the hemolymph nodes, accessory spleens and Kupffer liver cells, taking over the function of the spleen, but as yet no positive evidence has been produced to substantiate this theory. Splenectomy is not always followed by a decrease in the urobilin output. Robertson calls attention to the fact that those cases in which the urobilin excretion before splenectomy was high and which after the operation exhibited only a transient reduction or none at all did not show so much improvement in other respects as did those cases in which the urobilin output was permanently reduced.

Eppinger,<sup>40</sup> as already has been noted, asserts that in pernicious anemia and similar conditions the unsaturated fatty acids of the blood are increased. Splenectomy serves not only to reduce the unsaturated fatty acid content, but it also increases the cholesterol and total fat content of the blood. King<sup>35</sup> believes that the occurrence of highly unsaturated fatty acids is in some way intimately dependent upon the activity of the spleen, for he found that following splenectomy the iodine number of the fats of the blood is reduced and the production of icterus by hemolytic agents is made more difficult.

The most evident feature of the chemical pathology of the liver in pernicious anemia is the enormous deposits of hemosiderin which occur (Hunter<sup>64</sup>), and this fact substantiates the view that the hemolytic action takes place in the portal circulation. If the reverse were true, or if hemolysis occurred both in the portal and general circulation, one would expect to find iron deposits in the spleen increased, but such is not the case. (Mathes,<sup>65</sup> Hunter.<sup>64</sup>) The difference in the character of the deposits resulting from lysis and phagocytosis has been mentioned. (Muir and Dunn.<sup>54</sup>) That the Kupffer cells of the liver are in a state of increased erythrocyte phagocytosis in pernicious anemia has been shown by Moffitt.<sup>32</sup>

#### THE KIDNEYS IN PERNICIOUS ANEMIA.

But little work has been done with reference to the kidney chemistry in pernicious anemia. Thus far an abstract of a paper read by Christian<sup>66</sup> at Peter Bent Brigham Hospital is practically the only source of information on this phase of the subject. The phenolsulphonephthalein output even in severe cases was found to be normal, indicating no excretory insufficiency. If the urine of normal individuals who are receiving three meals a day is tested at intervals of two hours, decided variations in the volume and in the amount of solids present will be found. From figures thus obtained a curve may be plotted the contour of which is decidedly jagged (picket fence curve). In diseases of the kidneys the ability to vary the specific gravity and the output of various constituents is

diminished, in consequence whereof the curve loses its jagged appearance and flattens out. In pernicious anemia the specific gravity curves thus plotted become almost straight lines, while the curves of nitrogen elimination likewise show flattening, but to a less marked degree. Such curves point to the presence of functional kidney disturbances quite similar to those of chronic nephritis, and the appearance is fairly constant from day to day. After transfusion the functional curves become slightly more irregular. Pathologically the kidneys show no signs of nephritis. An increase in iron pigment, chiefly in the convoluted tubules, is about the only finding of note. Christian is working at present on the functional kidney curves obtained in pernicious anemia after various modes of treatment such as transfusion, splenectomy, arsenic, salvarsan, etc., and his results will doubtless be of much interest and value. The urine in pernicious anemia is usually of low specific gravity, and is pale from diminished pigment.<sup>67</sup>

#### GASTROINTESTINAL CONDITIONS IN PERNICIOUS ANEMIA.

Gastrointestinal disturbances are of peculiar interest in pernicious anemia for alimentary disorders have been and still are by many, considered the primary cause of the disease. Hunter,<sup>64</sup> as has been previously mentioned, regards the disease as an infection due to a specific glossitis, oral, gastric and intestinal sepsis. Stern<sup>65</sup> emphasizes the fact that periodically a soreness of the tongue and gums is noted, which is of importance from a diagnostic standpoint as one of the earliest symptoms. Mathes<sup>65</sup> also considers changes in the oral mucosa one of the earliest symptoms. Osler<sup>67</sup> says that pyorrhea alveolaris is found in every case of pernicious anemia. No special chemical agent is given as the cause for this condition, and indeed aside from the many references to stomatitis, little mention of the mouth is made.

In the stomach an absence of free HCl is a constant finding, so constant in fact, that in anemias where free HCl is present the pernicious type is ruled out. The combined acid also is extraordinarily low, being seldom over 10 in severe cases and more often it does not exceed 5. The achlorhydria in itself signifies faulty gastric digestion since pepsin is unable to act save in slightly acid media, but apparently in the majority of cases there is in addition more or less complete achylia gastrica. The significance of these conditions is a subject much debated. On the basis of postmortem and clinical observations von Nothnagel<sup>69</sup> concluded that the alimentary disturbance was the fundamental cause of pernicious anemia. Lewy<sup>70</sup> and Fenwick<sup>71</sup> agree with this view. On the contrary Baur and Weigle have advocated that the intestinal destruction is brought about by changes in the blood. Nolen<sup>73</sup> has reported two cases of pernicious anemia in which atrophy of the mucosa was present, and Quinke,<sup>74</sup> Henry and Osler,<sup>75</sup> and Brabazon<sup>76</sup> have each reported one case with similar gastric findings. Brabazon's case was not substantiated by microscopic examination. Kinnicutt,<sup>29</sup> in 1878, reported two cases of pernicious anemia both of which were autopsied. The stomach wall where it came in contact with the fluid contents of the stomach was in both cases thinner than normal. Microscopical examinations were made of sections from various regions of the stomach wall, and in selecting material care was used to obtain tissue which had not been in long contact with the gastric fluids.



Extensive areas of mucosa were found in which not a trace of gastric tubules could be seen. In some places, especially in the pyloric zone, remnants of tubules were found. A peculiar hyaline material was also seen taking in places the shape of the tubules and forming hyaline casts. In both cases there was a general dense small-cell infiltration of the mucosa such as is seen in inflammatory processes. That the atrophy was not due to postmortem effects is indicated by the fact that the more superficial parts of the glandular structure were least involved; greatest destruction took place in the deeper tissues. Kinnicutt<sup>29</sup> believed that the dense small-cell infiltration certainly could not be regarded as the result of impaired nutrition, and that in his cases at least this process strongly pointed to a dependence of atrophy upon an inflammatory process. He concluded that as a result of inflammation a primary atrophy of the gastric mucosa occurs, and that in this lesion is to be found an explanation of certain cases of pernicious anemia. Strausz<sup>72</sup> speaks of the coincidence of apepsia gastrica and pernicious anemia and holds that certain relations must exist between the disease and abnormal conditions of the intestinal mucosa. Some of the more recent autopsy findings apparently show that the mucosa is unaltered both in the stomach and in the intestine. Faber and Bloch<sup>71, 82</sup> found that providing postmortem changes are avoided by injection of formaldehyde, atrophic changes of the gastric and intestinal mucosae in pernicious anemia are often absent or slight. R. von Lippman<sup>77</sup> autopsied a case of pernicious anemia that clinically showed achylia gastrica. Especial care was used to guard against postmortem changes (the intestine was removed one hour after death), and in this case no evidences of atrophy of the mucosa and nothing of note, save extreme anemia of tissues, was found. Consequently he agrees that unless autopsy is quickly performed findings with reference to the mucosa in such cases are of little value. Whatever the pathological findings in such cases may be, it is certainly true that there are very marked alterations of the gastric secretions. The apparent lack of pepsin and inactivity of the acid-secreting glands seen clinically are rather hard to explain in the light of the normality of the mucosa claimed to have been found at autopsy. Whether or not normal peptic secretion goes on and the uniform lack of digestive ability is due to an antienzyme of some sort is a question well worth investigation. It must be borne in mind that the presence of NaCl, carbohydrates, and the products of protein digestion all tend to inhibit peptic activity.

Concerning the condition of the pancreatic-intestinal enzymes and hormones no information is afforded by the literature. Probably they are able to compensate for the digestive inability of the stomach, for were this not the case much more serious alimentary disturbances would be present than are usually found.

#### EXPERIMENTAL ANEMIAS.

We propose here not to enter into a detailed discussion of experimental anemias in general, but rather to enumerate a few of the methods by which anemias with a blood picture resembling that of pernicious anemia have been produced. Preeminent in this group is the experimental pernicious anemia produced by Seyderhelm<sup>9</sup> in healthy horses by the injection of filtered serum from a horse sick with the disease. The possibility of homologous sensitization in this case has already been discussed. Kumagai<sup>78</sup> showed that the erythrocytes of

sensitized animals upon second injection with the specific protein are less resistant to destructive agents of various kinds than is normally the case. Against saponin, however, he found the resistant force unchanged. Schittenhelm, Weichard and Grissamer<sup>2</sup> have shown that in artificial poisoning the hematopoietic organs are stimulated to great activity. Protein split products have the same stimulating effect and cause megaloblasts and megalocytes to appear in the peripheral blood in large numbers. The reduced erythrocyte resistance together with the bone marrow stimulation combine to give a picture very closely resembling in many cases idiopathic pernicious anemia. Schlecht and Schwenker<sup>79</sup> injected adrenalin in dogs and found that this drug caused a decrease in the number of reds, a decrease in the eosinophiles, and a decrease in the total hemoglobin content. Iwao<sup>14</sup> by repeated subcutaneous injections of p-oxyphenylethylamin was able to produce in guinea pigs an anemia with the characteristics of pernicious anemia both with respect to the blood picture and course; the number of erythrocytes and the total hemoglobin content were markedly reduced, while the hemoglobin content of the individual reds was relatively high. Poikilocytosis and polychromatophilia both occurred to a low degree, and both normoblasts and macrocytes were found. The number of eosinophiles was reduced, but there was a relatively high lymphocyte count. Iwao<sup>14</sup> compares the physiological action of p-oxyphenylethylamin with adrenalin in that both cause a rise in blood pressure due to the effect on the smooth muscle fibers. We have already discussed the intestinal source of this poison and pointed out the possibility of its being an etiological factor in pernicious anemia.

Buting<sup>80</sup> produced anemia in rabbits by the injection of ricin. After injection of the drug there was a leucopenia followed by leucocytosis, pyknosis and karyolysis as a rule. The red cells showed marked hemolysis indicated by hemoglobinuria and pigment accumulations in the spleen pulp, liver and lymph glands. Nucleated reds appeared in the peripheral blood stream, and their number depended first on the amount of toxin injected, and secondly, on the condition of the erythrogenetic tissue of the marrow. Buting's attempts to produce chronic anemia with ricin were only partially successful, because tolerance to the drug was rapidly established. In those cases in which the rabbits remained partially susceptible, an anemia was eventually produced with a blood picture identical with pernicious anemia and entirely different from that seen in the secondary anemia following hemorrhage. With saponin it was much easier to produce a chronic anemia since tolerance to it is less readily established. A reduction of the red count, nucleated reds in the blood stream, and pigment accumulations in the organs were all seen as was the case with ricin. Autopsy of animals rendered anemic in this way showed partial depletion of the mature bone marrow cells and necrosis of the remainder. There was marrow sclerosis together with diffuse hemorrhage. The liver showed central necrosis of the lobules together with marked turgescence of the veins and capillaries. From these findings Buting<sup>80</sup> concludes that the bone marrow throws nucleated reds into the circulation only when the mature erythrocytes at the periphery of the erythrogenetic center are destroyed by some toxin or depleted by hemorrhage. If there is extensive bone marrow injury, the groups of blood forming cells may be almost completely

replaced by scar tissue in which case the spleen becomes hematopoietic, and blood cells are formed in its sinuses. Unless large amounts of toxin are quickly thrown into the circulation, the bone marrow is uninjured; e. g., upon subcutaneous injection of ricin or saponin absorption is so slow that the toxin is saturated by cells in the circulation with resulting erythrolysis and the cells of the bone marrow are consequently uninjured.

#### SUMMARY AND CONCLUSIONS.

1. Pernicious anemia may be considered the result of a gastrointestinal disturbance leading to destruction of mucosa sufficient either to allow undigested proteins to leak through into the blood stream where their parenteral digestion liberates protein poisons, or to the absorption of known hemolytic toxins produced in the course of intestinal putrefaction such as, e. g., p-oxyphenylethylamin. In either case the poison has a destructive influence on the intestinal mucosa, as well as the more evident hemolytic effect, and this results in the establishment of a vicious circle.

2. Others consider the disease to be the result of some change in the lipid metabolism whereby large amounts of unsaturated fatty acids such as oleic are liberated.

3. Hypersplenism is a third explanation given as a possible cause for pernicious anemia. Stasis or occlusion of the splenic artery causes congestion of the sinus spaces and those erythrocytes which come in contact with the parenchyma are so changed as to be marked for destruction.

4. The clotting ability of blood in pernicious anemia is reduced, probably as a result of a reduction in the number of platelets.

5. There is an extreme oligocythemia and reduction in the total volume of blood. The hemoglobin content of the individual cells is relatively high.

6. The erythrocytes in pernicious anemia are more susceptible to such hemolytic agents as saponin. This is probably a result of the decrease in the cholesterol content of the cell envelope.

7. Specific proteolytic ferments may be present in the blood as appears to be the case in pernicious anemia of horses, and homologous sensitization, at least in the case of this animal, is possible.

8. The severity of the anemic blood picture is an expression of the difference in the rate of regeneration set against the rate of red cell destruction.

9. The only efficient method for determining the rate of destruction lies in quantitative estimations of pigment production, and this is best carried out by examination of the duodenal contents.

10. The basal metabolism is increased in pernicious anemia because of the excessive oxygen needs of nucleated reds. In spite of the low oxygen carrying ability per unit of blood, compensation may be effected by an increased heart rate and volume output.

11. Iron is deposited in large amounts in the liver, spleen, lymph glands, and to some extent in the kidney. The comparatively enormous deposits of iron in the liver indicate that erythrolysis occurs in the portal circulation.

12. Splenectomy lessens the severity of the disease by increasing the resistance of the reds to hemolytic agents. When the hemolymph glands and accessory spleens take on the splenic function a relapse occurs.

13. Liver changes are characterized chiefly by enormous deposits of hemosiderin.

14. Except in cases where there is hemoglobinuria, iron deposits in the kidney are slight and the amount of iron deposited is roughly proportional to the degree of hemoglobinuria.

15. In all cases of pernicious anemia there is achlorhydria, and in a large majority of cases achylia gastrica as well. Autopsies have given conflicting results as to the condition of the mucosa, and the apparent deficiency of enzymes may be due to the presence of inhibiting substances. This, however, is improbable.

16. By injections of hemolysins, such as saponin, experimental anemias can be produced in animals that are apparently identical both in the blood picture and course with true idiopathic pernicious anemia.

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## A REPORT OF TWO CASES OF FATAL HEROIN POISONING\*

BY WM. D. McNALLY, A.B., CHICAGO.

IN a review of the literature, I found reported only one case of fatal heroin poisoning,<sup>1</sup> with a possible second case in which the heroin was mixed with antipyrine. Poisoning with heroin, which did not produce death, is reported by Glasgow.<sup>2</sup> A woman 34 years old, took .05 gram of heroin hydrochloride. After twenty minutes she complained of a severe headache, and cramps extending to the lower extremities. The pulse was 100 to 110 and regular. The eyes were wide open, with a fixed staring gaze, and reacted to light. She was restless for two hours, and talked as if out of her right mind. Most of the symptoms were those of morphine poisoning. The poisoning reported by Trawick,<sup>3</sup> from a hypodermic injection of  $\frac{1}{12}$  grain, caused, a few minutes later, an irregular respiration, and the pulse almost ceased. The patient had the appearance of a person profoundly under the influence of an opiate, but recovered after the administration of strychnine, salt solution, and artificial respiration. In the report of Weinzieher,<sup>4</sup> a woman slept for 52 hours after taking .01 gram of heroin. The symptoms reported by Soles,<sup>5</sup> after a woman had taken .167 gram of heroin, were as follows: myosis, slow pulse (40), subnormal temperature, and cramps in the extremities. In a case of poisoning reported by Comar and Buvat<sup>6</sup> 2.8 grams was consumed in twenty-four hours by a patient resulting in a high degree of nervousness, feeble-mindedness, and a general wretched condition. The heart and kidneys were less active than normally. Harnack<sup>7</sup> claims that heroin is more poisonous to man than to animals, giving a lengthy discussion of the pharmacology of heroin. Dreser<sup>8</sup> found that the lethal dose for rabbits was 100 times the therapeutic dose, codein ten times the therapeutic dose; in other words, for rabbits codein is more toxic than heroin.† The pharmacological and therapeutical literature is voluminous, and it is only within the last five years that heroin has become of toxicological interest.

*Case No. 1.*—There has been opportunity for me to study material of a case of heroin poisoning, entered to the service of Dr. Goldsmith, Ward 22, of the Cook County Hospital. W. K., age 20 years, a salesman, was admitted in an unconscious condition, and died seven hours later. The physical examination was comparatively negative save for coolness of the body, a blue mottling of the abdomen and a weak and irregular heart. The pupils were contracted. The pulmotor was used for intervals of three-quarters and one-half hours respectively. The stomach was washed out with permanganate solution. The respirations were very slow being 13 per minute on admission to the hospital and after the first use of the pulmotor were 42. They fell down to 16 when the pulmotor was used again. Just before death they were 19 per minute. From the postmortem examination, (Dr. E. R. Le Count) the following anatomic diagnosis was reached: The pupils of the eyes were equal and dilated, the external surface of the brain presented no change. Upon sectioning the pons, medulla, and cerebellar hemispheres no gross alterations or evidence of disease was noted. There was an engorgement of the abdominal veins and marked

\*From Cook County Coroner's Laboratory, Chicago, Ill.

†Dreser found 0.1 gram of heroin and 0.1 gram of codein to be the lethal doses for rabbits. The conclusion derived was based on the therapeutic dose of heroin .001 gram, and the therapeutic dose of codein .01 gram. Using these values, he obtained  $.10 : .001 = 100$  for heroin.  $0.10 : .01 = 10$  for codein.

passive hyperemia and edema of the lungs; petechial hemorrhages in the epicardium and in the lining of the greater antrum of the stomach; dilatation of the mitral ring; persistent lymphoid tissue in the thymic body; hyperplasia of the lymph nodes in the spleen, of the lymph glands, of the solitary follicles of the stomach, small and large bowel, and of the Peyer's patches; hyperplasia of the aorta; slight chronic catarrhal prostatitis; left obliterative fibrous pleuritis. In an examination of the urine, (made by Prof. W. S. Haines for Dr. E. R. Le Count), albumin and sugar were found to be present in considerable quantities.

The results of the chemical examination of the organs are shown in Tables I and II.

TABLE I.

LAB. NO.	ORGAN	WEIGHT IN GRAMS	WT. USED IN GRAMS	ALKALOID FOUND IN GRAMS	TOTAL ALKALOID FOUND IN GRAMS
259	Brain	1236	200	None	None
260	Stomach contents	454	200	.0414	.0939
261	Intestines	1315	200	.0163	.1071
262	Liver	1480	171	.0018	.0155
263	Intestinal contents	1020	300	.0020	.0068
264	Kidney	125.5	100	.0007	.0009
265	Spleen	190	190	Trace	Trace
266	Stomach	349	120	.0256	.0744
267	Preservative on 261, 263, and 266	2250	300	None	None
268	Preservative on Kidney, Liver and Spleen	1040	200	.0007	.0036
					3022

The alkaloid extracted from the liver, kidney, spleen, the preservative in which these organs were held, the intestines, and intestinal contents gave all the reactions for morphine. The alkaloid extracted from the stomach and stomach contents responded to the tests for heroin.

*Case No. 2.*—Mr. L. D., age 32, entered the service of Dr. Portis, Ward No. 24, of the Cook County Hospital, May 25, 1913. A transcript taken from the clinical record gave the following:

Patient in a comatose condition, respiration slow and grouped over periods of one and one-half minutes, with intervening periods of dyspnea lasting over one minute. Body of patient cold. Skin is dry and lobes of ears are cyanotic. Pulse is slow, small, regular, and soft. Pupils are pinpoint, conjunctivæ injected. No paralysis. Lips, mouth, and throat are negative. Heart normal, abdomen lax, no dullness. Extremities flaccid, reflexes present. From the postmortem examination (Dr. E. R. Le Count) the following anatomic diagnosis was made:

Passive hyperemia of the lungs, of the brain, and of the retroesophageal tissues; marked edema of the lungs, jejunum and ileum; marginal emphysema of the lungs; slight hyperplasia of the spleen. The lungs did not collapse when the pleural cavities, containing a large amount of fluid, were opened. The pulmonary artery and its branches were empty. The heart was loose and flabby. The inner surface of the calvarium was of a bluish gray. A large amount of cerebrospinal fluid was present, being very abundant in the sulci of the vertex of the brain where the convolutions are widely separated. There were no alterations in the fourth ventricle. On the surfaces made by sectioning the brain in the usual way, there was no gross disease. The blood from the heart gave a strongly positive Wassermann reaction.

A portion of the intestines, stomach, and stomach contents were submitted to me for examination. An analysis showed that the alkaloid heroin had been taken by the deceased. The examination was not as thorough as in the first case, because the material obtained was not as abundant. The chemical examination, results of which are shown in Table II, indicates that heroin can be recovered from the stomach and stomach contents unchanged. The Stas-Otto process was used in extracting the alkaloid from the tissues in the two cases reported.

TABLE II.

LAB. NO.	ORGAN	WEIGHT IN GRAMS	WT. USED IN GRAMS	ALKALOID FOUND IN GRAMS	TOTAL ALKALOID
393	Intestines	806	200	.0034	.0137
394	Preservative on 393 and 395	1036	300	.0008	.0027
395	Stomach	281.4	200	.0153	.0215
396	Stomach contents	47.0	47.	.0057	.0057
					.0436

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# LABORATORY METHODS

## ANIMAL INOCULATIONS SUPPLEMENTARY TO AN INTRODUCTORY COURSE IN PATHOGENIC BACTERIOLOGY\*

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THE essential feature of the introductory course in Pathogenic Bacteriology in Leland Stanford Junior University is the inclusion among the required student exercises of numerous animal inoculations and immunizations, together with introductory exercises in aseptic surgical technic. A weekly program of the course is given in Schedule I; the inoculations, immunizations, etc., in Schedule II.

The equipment for these exercises consists of four small rooms, in easy access to the microscopical laboratory, serving as: animal room, autopsy room, inoculation room, and operating room. The cost of animals, feed, etc., is about \$5 per student, the students working in groups of two.

No objection to this work has been made by local antivivisectionists.

### SCHEDULE I. WEEKLY PROGRAM.

- 1st week—Media, air colonies, plating.
- 2nd week—Molds, yeasts, chromogens.
- 3rd week—Saprophytic, putrefactive.
- 4th week—Disinfectants, biological defenses.
- 5th week—Animal diseases.
- 6th week—Pyogenic, subpyogenic.
- 7th week—Anacrobies, toxemias.
- 8th week—Spirilla.
- 9th week—Midsemester practical examination.
- 10th week—Typhoid, dysentery.
- 11th week—Colon, enteritis (mixture of unknown).
- 12th week—Acid-fast, higher bacteria, blastomycetes.
- 13th week—Spirochetes, protozoa.
- 14th week—Final practical examination (includes at least one autopsy).

### SCHEDULE II. INOCULATIONS, IMMUNIZATIONS, AUTOPSIES, ETC.

#### *3rd Week.*

#### METHODS OF INOCULATION, TESTS OF COMMON SAPROPHYTES.

TUESDAY.—Each group to inoculate four animals with pure cultures obtained from water, milk, decaying vegetable and animal matter, etc.

- 1. *Rabbit*: Intravenously, 2 c.c. 24 hr. broth, No. 1.
- 2. *Guinea Pig*: Intraperitoneally, 1 c.c. 24 hr. broth, No. 2.
- 3. *Pigcon*: Intramuscularly, 1 c.c. 24 hr. broth, No. 3.
- 4. *Rat*: Subcutaneously, 0.5 c.c. 24 hr. broth, No. 4.

Note clinical symptoms daily for two weeks. No autopsies.

#### BACTERIAL DISTRIBUTION, NORMAL ANIMAL BODY.

THURSDAY.—5. *Large Rabbit*: Etherize; with aseptic precautions ligate (i) trachea, immediately above clavicle, (ii) trachea, immediately below larynx, (iii) esophagus, middle of

\*Presented before the Pedagogical Section, Society of American Bacteriologists, New Haven, Conn., Dec. 28, 1916.

neck. Wash out with sterile NaCl-Sol.: (a) trachea, below ligatures, (b) trachea, between ligatures, (c) trachea, above ligatures, (d) pharynx, (e) esophagus, above ligature, (f) pleural cavity, (g) peritoneal cavity. With capillary pipettes draw samples of: (h) heart-blood, (i) bile, (j) urine, (k) gastric contents, (l) duodenal contents, and (m) contents of large intestine. Plate out one loopful of each sample thus obtained.

#### 4th Week.

##### BIOLOGICAL DEFENSES.

MONDAY.—6. *Rabbit*: Draw 10 c.c. blood, aseptic precautions, carotid artery. Allow blood to coagulate, store in ice chest overnight. On Tuesday study bactericidal power (*B. typhosus*) of: (a) serum, (b) heated serum (60° C., 30 min.).

TUESDAY.—7. *Rabbit*: Kill by blow on back of neck, open abdomen, aseptic precautions, aspirate bile, and urine. Remove stomach contents, moisten with sterile salt-solution, centrifuge free from undigested food material. Test bactericidal power (*B. typhosus*) of: (c) urine, (d) bile, (e) gastric fluid.

WEDNESDAY.—8. *Large Rabbit*: Inject, late in afternoon, 10 c.c. 5% aleuronat into each pleural cavity. On Thursday kill rabbit by blow on back of neck, exsanguinate from carotid, open abdomen, aseptic precautions, and aspirate pleural exudate through diaphragm. Make smears, stain by Wright's method. Defibrinate, mix one sample with bacteria or spores (oral directions), incubate, make smears at end of 15 min., 30 min., and 1 hour.

#### 5th Week.

##### INTRODUCTION TO PATHOGENICITY, AUTOPSY TECHNIC.

MONDAY.—9. *Guinea Pig*: Subcutaneously, 1 loopful 48 hr. broth, *B. anthracis*. Note clinical symptoms daily. Expect autopsy Wednesday. Plate out heart blood. Identify recovered microorganism. Make smears from heart blood, liver, spleen, and kidney. Histological sections will be made for you.

10. *Pigeon*: Intramuscularly, same dose, *B. anthracis*. Follow clinical symptoms daily for one week. No autopsy.

11. *Male Guinea Pig*: Intraperitoneally, 1 c.c. 48 hr. broth, *B. mallei*. Note clinical symptoms daily for 10 days. No autopsy.

THURSDAY.—12. *Rabbit, Guinea Pig, or Pigeon*: Subcutaneously, 1/10 loopful 24 hr. broth, *B. pleurisepticus*. Expect autopsy Friday. Recover and identify microorganism from heart blood. Make smears from heart blood, liver, spleen, and kidney.

FRIDAY.—Inoculate three flasks, special broth, *B. diphtheriae* (Park No. 8). Incubate 10 days.

#### 6th Week.

##### PYOGENIC, SUBPYOGENIC.

MONDAY.—13. *Rabbit*: Intravenously, 1/20 c.c. 48 hr. broth, *S. aureus*. Note clinical symptoms daily. Expect autopsy in about four days. If animal does not die, etherize on Friday. Plate out heart blood, and material from abnormal areas in heart muscle, kidney, etc. Make smears from abnormal areas. Histological sections will be made for you.

14. *Rabbit*: Subcutaneously, 1/4 c.c. 24 hr. CaCO<sub>3</sub> broth, *S. pneumoniae*. Expect autopsy Tuesday. Recover and identify microorganism from heart blood, using special media. Make smears from heart blood, liver, kidney, and spleen.

WEDNESDAY.—15. *Small Rabbit*: Intraperitoneally, 4 c.c. mixed sputum. Expect autopsy Thursday. Plate out heart blood, using special blood-agar. Identify microorganisms isolated.

#### 7th Week.

##### TOXEMIAS, ANAEROBES.

MONDAY.—Filter one special 10-day culture *B. diphtheriae*. Test sterility of filtrate.

TUESDAY.—Centrifuge and wash the bacilli in one special 10-day culture *B. diphtheriae*. Suspend washed microorganisms in original volume, physiological saline.

16. 250 gm. *Guinea Pig*: Subcutaneously, 1/4 c.c. special 10-day culture, *B. diphtheriae*.

17. 250 gm. *Guinea Pig*: Subcutaneously 1/4 c.c. special 10-day culture, *B. diphtheriae*; followed by 1/10 c.c. diphtheria antitoxin, intraperitoneally.

18. 250 gm. *Guinea Pig*: Subcutaneously 1/4 c.c. filtrate, from same culture.

19. 250 gm. *Guinea Pig*: Subcutaneously 1/4 c.c. suspension, washed *B. diphtheriae*. Note clinical symptoms daily for 7 days. If animals die, make Loeffler's blood serum cultures from heart blood, and site of injection. Note conditions of internal organs, particularly the adrenals, stomach and peritoneal surfaces.

20. *Guinea Pig*: Subcutaneously,  $\frac{1}{4}$  c.c. 24 hr. broth, *B. edematis*. Note general and local symptoms daily. If death results, make smears from local lesions, liver, and heart blood.

8th Week.

#### SPIRILLA.

TUESDAY.—21. *Pigeon*: Intramuscularly, 1 c.c. 24 hr. broth, *S. cholerae*.

22. *Pigeon*: Intramuscularly, 1 c.c. 24 hr. broth, *S. metchnikovii*. If death results, isolate and identify microorganisms from heart blood. Make smears from heart blood and site of inoculation.

6th-10th Week.

#### AGGLUTINATING SERA.

23. *Rabbit*: Six repeated injections, three to five day intervals, 1 c.c. 24 hr. broth, *B. typhosus* or *B. dysenteriae*. Make first two injections subcutaneously with killed cultures ( $60^{\circ}$  C., 1 hr.); second two intraperitoneally, and last two intravenously with living cultures. Eight to ten days after the last injection draw serum, carotid artery, aseptic precautions. Determine: titer, specificity.

7th-12th Week.

#### TUBERCULOSIS.

24. *Guinea Pig*: Intraperitoneally, 1 c.c. suspension, *B. tuberculosis*.

25. *Guinea Pig*: Intraperitoneally, 1 c.c. tuberculous sputum. Keep animal in special cage. Examine twice weekly. Etherize about four weeks later. Histological sections will be made for you.

10th-11th Week.

#### ANAPHYLACTIC REACTION.

26, 27. 200 gm. *Guinea Pigs*: Subcutaneously,  $\frac{1}{40}$  c.c. goat serum: Test guinea pigs 14 to 18 days later, 3 c.c. goat serum, injected through ball of hind foot. Make similar injection into a normal control. If animals die, open thorax and determine condition of lungs.

10th-13th Week.

#### HEMOLYSIS.

28. *Rabbit*: Four repeated injections, three to five day intervals 1 c.c. 25% suspension goat corpuscles (suspensions will be prepared for you). Draw serum, marginal vein, eight to ten days after the last injection. Test: Inactivation, reactivation with guinea pig complement.

13th Week.

#### TRYPANOSOMES, SPIROCHETES.

MONDAY.—29. *White Rat*: Subcutaneously,  $\frac{1}{4}$  c.c. rat's blood, *T. lewisi*.

30. *White Rat*: Subcutaneously,  $\frac{1}{4}$  c.c. rat's blood, *S. recurrentis*.

Examine blood daily. Make smears at height of infection. No autopsy.

4th-12th Week.

#### ASEPTIC SURGICAL TECHNIC.

31, 32. *Large Rabbits*: Each group to do two laparotomies. Among the operations recommended are: (a) Nephrectomy; (b) Splenectomy; (c) Ligation of inferior vena cava.

## NOTE ON TECHNIC WITH FRIDERICIA APPARATUS

BY MILES J. BREUER, M.D., LINCOLN, NEB.

IN the *Journal of Laboratory and Clinical Medicine*, April, 1916, appears a description by J. J. R. Macleod of the apparatus and technic for determining the alveolar  $\text{CO}_2$  by the Fridericia method. In following this technic, I was impressed with a considerable variation in the results of determinations in close succession and on the same person, on myself for instance. The inaccuracy was traced to the following cause. After the enclosed air is shaken with the

alkali, being shut in at both ends by the stopcocks, Macleod's directions read that the stopcock B should be turned into such a position that the water in which the apparatus is immersed, will run into the graduated tube. (This would actually be the position of Fig. III, and not that of Fig. II as stated there, the latter being probably a typographical error.)

When the stopcock B is turned into such a position, so that the water will run up into the graduated portion, it will usually carry with it a bubble of air, which has been held inside the stopcock. When the surplus alkali is allowed to run out, the stopcock is emptied, and as it is turned off, the air is enclosed in it. Then, when the water runs up into the graduated portion, the bubble is carried with it. This is obviated by the following modification of the technic as given by Macleod.

After shaking up the enclosed air with the alkali, immerse the apparatus up to the stopcock A in a cylinder of water, and turn the stopcock into position as shown in Fig. II, so that the water will run first into the ungraduated side of the instrument, filling the interior of the stopcock with water. Then the stopcock is turned into the position shown in Fig. III, so that the graduated side communicates with the water outside.

With this change in the technic, I have been able to obtain very consistent and accurate results.

## CYANIDE POISONING IN A CASE OF TYPHOID FEVER

BY LUDVIG HEKTOEN, M.D., WALTER S. HAINES, M.D., AND  
VICTOR C. VAUGHAN, M.D.

THIS report concerns a man, about 30 years old, previously in good health, who was in the early stages of typhoid fever and receiving the usual treatment. At 3:30 P.M. on the second or third day after he had taken to bed he received a particular capsule, which there is reason to believe contained potassium cyanide, and about half an hour later he was seized with a convulsion and became unconscious. The details are given below as taken from the nurse's chart.

TIME.	TEMP.	PULSE.	RESP.	NOTES.
Dec. 5, 1 A.M.	102.8	99	..	
" " 3 "	102.6	98	..	
" " 6 "	101.4	98	..	Slept 2½ hours during night.
" " 9½ "	99.8	78	..	
" " 12 M.	100.8	80	..	
" " 2¾ P.M.	103.4	96	22	
" " 3½ "	....	..	..	Receives a particular capsule; vomits a little; at 4 o'clock he is seized with a convulsion and becomes unconscious; the arms are bent and drawn up, the hands clenched, the legs rigid, the eyes open and staring, the jaws locked, the face pale and somewhat cyanosed, and a peculiar humming sound is produced in the throat. The pulse weak and fluttering. Digitalin gr. ⅓ <sub>100</sub> and strychnine gr. ⅓ <sub>40</sub> and nitroglycerine gr. ⅓ <sub>100</sub> .

	TIME.	TEMP.	PULSE.	RESP.	NOTES.
Dec. 5,	4 $\frac{3}{4}$ P.M.	104.2	132	..	Patient quieter.
" "	5 $\frac{1}{4}$ "	103.4	120	..	$\frac{1}{8}$ gr. morphine hypodermically.
" "	6 $\frac{3}{4}$ "	102.6	108	11	$\frac{1}{8}$ gr. morphine again.
" "	7 $\frac{3}{4}$ "	101.8	106	16	
" "	8 $\frac{3}{4}$ "	102.	104	..	Still rigid; morphine gr. $\frac{1}{8}$ hypodermically.
" "	9 $\frac{3}{4}$ "	101.6	104	18	Seems more relaxed.
" "	10 $\frac{3}{4}$ "	101.8	106	16	
" "	11 $\frac{3}{4}$ "	102.6	108	..	Strychnine gr. $\frac{1}{100}$ hypodermically.
Dec. 6,	12 $\frac{3}{4}$ A.M.	102.6	104	16	
" "	1 $\frac{3}{4}$ "	103.4	108	18	Arms and legs rigid.
" "	2 $\frac{3}{4}$ "	103.4	108	16	Morphine gr. $\frac{1}{8}$ hypodermically.
" "	3 $\frac{3}{4}$ "	104.4	108	16	
" "	4 $\frac{1}{2}$ "	....	..	..	Morphine gr. $\frac{1}{8}$ , strychnine gr. $\frac{1}{40}$ hypodermically.
" "	5 $\frac{3}{4}$ "	105.	112	12	Respirations shallow.
" "	7 "	105.6	134	16	Rigid; jerks arms and legs at intervals.
" "	7 $\frac{3}{4}$ "	104.6	120	12	Morphine gr. $\frac{1}{8}$ , strychnine gr. $\frac{1}{40}$ hypodermically.
" "	8 $\frac{1}{2}$ "	105.2	138	..	
" "	10 $\frac{1}{2}$ "	106.6	140	26	
" "	11 $\frac{1}{2}$ "	105.6	136	24	
" "	12 $\frac{1}{4}$ P.M.	106.	140	32	Respiration shallow.
" "	1 "	106.	144	30	Leucocytes 3-4000 per c.mm.
" "	2 "	....	..	..	Delirious; nitroglycerine $\frac{1}{100}$ gr.
Dec. 6,	2 $\frac{3}{4}$ P.M.	107.8	Uncountable.	56	
" "	3 $\frac{3}{4}$ "	106.5	"	45	
" "	4 "	....	..	..	A particular capsule.
" "	4 $\frac{1}{2}$ "	106.2	150	38	
" "	5 $\frac{1}{4}$ "	104.6	148	30	Very restless.
" "	7 "	104.2	140	48	Convulsion—Death.

Soon after death the body was injected with a fluid containing formaldehyde and placed in a vault. The postmortem examination was made 23 days later. The body was in an excellent state of preservation and there was what was regarded as "some superficial bruises of the skin of the legs." There was well marked swelling of Peyer's patches and lymph follicles in the small intestines, most marked nearest the ileocecal valve. The spleen was enlarged and deep red in color. The liver was normal in size and showed on microscopic examination many typical areas of focal necrosis. The right chest cavity was obliterated by firm adhesions and the posterior parts of the lower lobes of both lungs were deeply congested and in part solid. The pia of the brain was congested. All the other organs appeared to be normal.

The chemical analysis (by Haines and Vaughan) gave small amounts of strychnine in the contents of the stomach and in the liver and definite indications of cyanide in the stomach contents.

Of the symptoms of cyanide poisoning, the slowing of the respiration observed for some hours after the first convulsion is highly significant.

## IMPROVED TEST FOR INDICAN IN URINE\*

BY FRITZ C. ASKENSTEDT, M.D., LOUISVILLE, KY.

THE clinical significance of indicanuria is still a subject of much confusion largely because the clinical tests for indican in common use are unreliable. This will be made apparent by even a brief consideration of the chemical changes to which indican is subject during the performance of the tests. By oxidation two molecules of the colorless indican ( $C_8H_7NSO_4$ ) are converted into one of indigo-blue ( $C_{16}H_{10}N_2O_2$ ) which by further exposure to oxygen is as readily reduced to the reddish yellow isatin ( $C_8H_5NO_2$ ). Since the estimation of indican is always made on the amount of blue color obtained after indican has been converted into indigo-blue, it is of utmost importance to the accuracy of the test that all of the indican present be converted to indigo-blue, and that this, as soon as formed, shall be protected from further oxidation. Chloroform, in which indigo but not indican is soluble, serves to protect the indigo-blue from further chemical action. While the ordinary tests for indican are based on the above fundamental facts, grossly inaccurate estimates very often result from misuse or neglect of the following technical points:

1. *Proper Selection and Use of Oxidizing Agent.*—Even with the most thorough mixing of the oxidizing agent with the urine, the oxidation of all the molecules of indican contained in the urine does not take place simultaneously but progresses quantitatively, which renders the molecules of indigo-blue first formed liable to conversion into isatin before all the indican has been oxidized. The rapidity with which these processes of oxidation occurs depends upon the energy of the oxidizing agent, its concentration, and the temperature of the mixture. It is apparent that with an energetic oxidizer, such as potassium permanganate, the changes are much less under control than when a less energetic substance, as ferric sesquichloride, is employed. The practice of adding a few drops of a solution of potassium permanganate to a mixture of urine and hydrochloric acid has been found a faulty technic, because in addition to the disadvantages of an energetic oxidizer, there is, at least for a moment, an uneven distribution of the oxidizer, thereby overcharging a part of the urine, with the result of rapid isatin formation. An oxidizing agent of only moderate energy and with the largest possible surface of distribution is, therefore, to be preferred.

2. *Temperature.*—An objection to a low grade oxidizer, as the perchloride of iron, is the prolonged shaking with chloroform for extraction of the indigo, made necessary by the tardy action of the reagent. The simple direction to shake the mixture a few times and then set it aside for precipitation of the chloroform is entirely inadequate. The necessary duration of this process of extraction with chloroform is a serious objection to a general adoption and correct usage

\*For helpful suggestions in the development of the tests for indican I wish to acknowledge indebtedness to Dr. Clifford Mitchell, of Chicago.

of such a reagent, but the period of shaking can be materially shortened by raising the temperature of the urine to 130 to 140 degrees F. When the permanganate is to be used, warming the urine simply increases the perplexities.

3. *Extraction of Indigo*.—Chloroform seems the most satisfactory agent. It should be added to the urine and well mixed with it before the oxidizer is added, to insure immediate extraction of the molecules of indigo first formed. Chloroform is capable of holding only a small amount of indigo in solution, and therefore soon becomes saturated when shaken with a mixture rich in this ingredient. A common fault of most clinical tests for indican is that the quantity of chloroform added is too small, and, as has already been intimated, the duration of the extraction of indigo entirely too short. These two defects can not be too strongly emphasized.

4. *Concentration of Urine*.—Practically all urines contain indican. The detection of indican is, therefore, of no more value than the revealed presence of urea or phosphates. Qualitative tests for such normal ingredients of the urine are of value only if standardized for a definite concentration, as well as quantity, of urine. A reaction that is normal to a urine of a specific gravity of 1020, may prove decidedly abnormal to a urine of 1010. A uniform concentration or dilution of the urines to be tested is accordingly a prerequisite to a practical qualitative test for indican.

5. *Contaminating Substances*.—It has been positively demonstrated that certain gases in the laboratory, notably formaldehyde, interfere with the conversion of indican to indigo. For this reason the laboratory should be properly ventilated and all utensils duly cleansed before using.

The qualitative test for indican described below is a simplified procedure of the writer's quantitative test published in the *New York Medical Journal*, June, 1912, and is offered to those practitioners who can not afford the twelve minutes necessary for a quantitative determination.

Dilute the urine until it has a sp. gr. of 1005. For example, if urine shows a sp. gr. of 1017, dilute five parts of urine with twelve parts of water; if its sp. gr. is 1021, dilute five parts of the urine with sixteen parts of water, etc., corrections being made for temperature. An exception is made for diabetic urine, which is diluted until its urea content is 0.5 per cent. Place 10 c.c. of the diluted urine in a test tube and warm over a flame until the lower end of the tube begins to feel hot to the hand. Then add 8 c.c. chloroform and mix by shaking a few times. Ten cubic centimeters of a solution of 0.4 per cent perchloride of iron in concentrated hydrochloric acid (Obermeyer's reagent) is now added, and, with the tube duly stoppered, quickly extract the indigo by shaking the tube two minutes, holding it in a horizontal position. By releasing the stopper once or twice during the procedure, squirting will be prevented. After this, let the chloroform fall to the bottom of the tube, then pour off most of the supernatant fluid, fill the tube nearly full with water, invert it a few times to wash the chloroform, and let it again precipitate in the tube. If indican is normal in amount, the chloroform will remain white or show a mere trace of blue. Any increase in blue exhibits a proportionate excess of indican. The

reading should be made as soon as the chloroform is precipitated, for on standing, the chloroform slowly becomes transparent, with an increase in the shade of blue when present.

Years of experimentation have demonstrated the accuracy and clinical value of this test.

## THE DIAGNOSIS OF MALIGNANT TUMORS BY PARAFFIN SECTIONS OF CENTRIFUGED EXUDATES\*

BY F. S. MANDLEBAUM, M.D., NEW YORK CITY.

AS an aid in the identification of tumor cells or minute fragments of tumor tissue in pleural or ascitic fluids, an examination of the sediment by the histological methods employed in routine tissue work has given excellent results for many years and has supplanted the examination of stained smears prepared from the fresh sediment.

Briefly stated, the technic of this simple method is the following:

1. The sediment is obtained in large conical tubes by means of the centrifuge. A small quantity of fluid may prove sufficient, but where large amounts have been aspirated at least 500 c.c. should be centrifuged.

2. Carefully decant the supernatant fluid, or better still, remove the fluid with a pipette and large rubber bulb.

3. Cover the sediment with a deep layer of 10 per cent formalin which must be added by means of a pipette or gently poured into the tube to avoid disturbing the sediment.

4. After six to twelve hours, depending upon the amount of sediment, carefully remove the formalin and replace by alcohol 95 per cent for twenty-four hours.

5. Remove the disc of fixed and hardened sediment by means of a thin knife blade. Transfer through absolute alcohol and chloroform into paraffin in the usual manner, trimming down the block if necessary.

6. Cut, mount and stain sections as in routine histological examinations.†

Microscopic examination of the sections obtained by this simple procedure reveals tumor cells when present, or even tiny fragments of tumor tissue sufficient to establish the diagnosis of a malignant growth. If stained smears made from the fresh sediment are compared with the sections, the advantages of the method just described are apparent.

An accurate diagnosis of malignant tumors of the lung and pleura or of abdominal organs accompanied by ascites has been made by this method in a considerable number of cases, the diagnosis being confirmed subsequently by the clinical picture or by autopsy. A detailed account of these cases with the significance of budding cells, mitotic figures and other cytologic criteria is in preparation.

\*From the Pathological Department, Mount Sinai Hospital, New York City.

†Several years ago, in the examination of a case of actinomycosis, the writer succeeded in obtaining excellent sections from a drop of pus which was fixed in formalin and embedded in celloidin: *Proc. New York Path. Soc.*, 1901, 178.



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## EDITORIALS

### *The National Research Council*

THE National Academy of Science was chartered by Congress in 1863, and it is the only scientific body in this country possessed of a Congressional charter. In this charter it was stated that one of the purposes of this organization would be to advise Congress and the President concerning scientific matters. From its foundation up to recent times, Congressional Committees occasionally consulted the Academy concerning scientific subjects. When Mr. Wilson became President, there were vacancies in three scientific bureaus and he asked the National Academy of Science to nominate men for these positions. This was done, and the appointments were made from the nominees. In April, 1916, President Wilson asked the National Academy of Science to appoint a committee, to be known as the National Research Council, whose chief function would be to encourage scientific research in this country, and to advise the Government concerning scientific needs. In accordance with this request, the Council was appointed and held its first meeting in September, 1916. The Council numbers more than forty, and the work since the first general meeting has been done by an Executive Committee of ten. The men on this Committee have served without financial recompense, have met twice a month, with

occasional extra meetings, and have attempted to correlate the scientific work in educational institutions, in special research laboratories, and in industrial science. Special committees have been appointed to undertake this work in different departments of science.

The rapid approach of war has led the Council to concentrate its efforts upon war problems. While the Council covers the whole field of science, we are specially interested in that part of it concerned with medicine and hygiene. Among the problems that have been formulated, and at which men are now at work, we may mention the following:

1. *Antitoxins*.—There are ten or twelve commercial firms preparing diphtheria and tetanus antitoxins. Under the present law, all of these are working under the supervision of the Public Health Service. All manufacturers of biological products must be licensed by the Federal Government, and the license is issued only after inspection. Besides this, the Public Health Service goes on to the market at least once a month, buys these antitoxins, and ascertains experimentally whether or not they come up to standard. It seems that we can rely upon commercial houses to supply diphtheria and tetanus antitoxins. In this connection it is well to know that the British Army surgeons have come to the conclusion that the protective dose of tetanus antitoxin need not exceed five hundred units.

Flexner, in charge of the Rockefeller Institute, has undertaken to become responsible for the preparation and supply of serums for the treatment of pneumonia, dysentery, and meningitis. Experience on the Texas border has shown that the serum prepared in the Rockefeller Institute for the treatment of pneumonia of type No. 1 is highly efficient. The data concerning the efficiency of dysentery serums are not altogether satisfactory. There has been more or less dysentery—much more than there should have been—on the Texas border. Flexner has undertaken the responsibility of the preparation and supply of this serum. In this connection, it may be well to state that Dakin reports that Dale has prepared a mixture of emetin with bismuth iodide which may be given by the mouth and which has proved of marked service in dysentery.

Major Craig has found that the commercial serum obtainable is not reliable in the treatment of meningitis. Fifty per cent of the cases of this disease treated with this serum ended fatally. On the other hand, of ten cases treated with the Flexner serum, only one died, and this man was practically moribund when received at the hospital. Flexner has agreed to take the responsibility for the preparation and supply of this serum.

2. *Vaccines*.—McCoy of the Hygienic Laboratory will take the responsibility of securing from commercial houses and testing smallpox vaccine. Some important researches on various points connected with smallpox vaccination are being carried out in the University of California. Special attention is being given there to the intradermal method. This produces only a small pustule, thus decreasing the probability of dangerous infection, and at the same time seems to give complete immunity. The possibility of using the purified vaccine of Noguchi is being investigated.

There seems to be good reason for having all the typhoid vaccine prepared in one laboratory and under one direction. This secures uniformity. Colonel Arthur, in charge of the Army Medical Corps in Washington, reports that the laboratory of that school will be prepared to furnish enough typhoid vaccine to vaccinate five million men as fast as they can be enlisted. Information from our allies in Europe concerning polyvalent vaccines is being sought.

3. *Poisonous Gases*.—Several chemists are already at work collecting material already known, and making further investigations concerning the best methods of neutralizing the poisonous gases that have been used in the European war. The gases which have been most used are chlorine, bromine, hydrocyanic, carbon monoxide, and an arsenical gas.

4. *Excreta*.—Major Craig thinks that while, on the whole, methods of disposal of excreta at present in use in our Army are satisfactory, improvement can be made. In permanent and semi-permanent camps incinerators give good results. The possibility of mixing some chemical with the fecal matter in order to hasten its destruction is under consideration.

5. *Drinking Water*.—There seems to be no difficulty in securing safe water in the large camps. The Lyster hypochloride bag has been used on the border and on the incursion into Mexico, and has proved quite satisfactory. There is much need, however, of some tablet or ampul which the soldier may carry and which he may drop into his canteen when he is compelled to fill it from doubtful or unknown sources. There have been many cases of dysentery in Mexico and along the Texas border due to taking water from doubtful sources, especially when men are on scout duty, or when, for any reason, they are not within reach of the general water supply of the camp. It is expected that valuable information will be secured along this line from those who have been in Europe.

6. *Rations*.—It seems to be the general opinion among army medical men that the present ration is well balanced and is satisfactory. We understand that in the Mesopotamian campaign compressed yeast has been issued in order to supply vitamins. It has been suggested that since cranberries keep so easily, there might be a ration containing this valuable and delectable ingredient. The whole question of the food supply of the nation and its distribution is under consideration by the Council. We have such men as Taylor, Lusk, and Folin and others who are most competent to advise along this line, and their advice should be sought and followed before we really feel the necessity of regulating food distribution and consumption.

7. *Preserved Foods*.—The Canning Association has placed in the hands of the Harvard Medical School the sum of \$20,000 a year to be used by Rosenau in studying their products. Rosenau is working under a Committee selected by the National Research Council. This work will include everything pertaining to preserved foods, the possibility of their contamination and of food-poisoning resulting therefrom.

8. *Soldiers' Clothing*.—So far there has been no important criticism of the present clothing, nor suggestions concerning improvement. Color, weight, durability, imperviousness to moisture, are some of the factors that must be taken into consideration.

9. *Infected Wounds*.—Our veteran surgeon, Keen, is preparing a report upon knowledge acquired during the present war in the treatment of infected wounds. Surgeons who have been to the war zone in Europe are requested to communicate with Dr. Keen.

10. *Shock*.—Much good work has been done, and some of the best of it by American investigators on shock. We have such authorities as Porter, Crile, Cannon, Janeway, and others, and it is expected that such men will collect and arrange for service all of the best information along this line.

11. *First-Aid Dressings*.—Whether the first-aid package carried by the American soldier now can be improved upon is a question. There are rather complicating statements from abroad concerning the use of iodine in first aid. So far as it can be ascertained, it seems that iodine was at first used too abundantly and rather ignorantly, and that it did more harm than good. Further information along this line is desirable.

12. *Fatigue*.—It is highly desirable that some method can be found by which, on the examination of recruits, the man who is likely to suffer from heart-strain or from exhaustion should be recognized and should be assigned to the lighter duties. A man easily fatigued is not only unfit for the fighting line, but is a direct detriment to his companions on that line. Several prominent physiologists and clinicians are at work on this subject.

13. *Occupational Diseases*.—Edsall of the Harvard Medical School has been asked to associate with himself such other experts on this subject as he may need, and to investigate the matter. We are especially concerned with those diseases which afflict munition manufacturers.

14. *Veneral Diseases*.—This is one of the biggest problems with which the Council has been confronted in the medical line. At present every recruit is given the Wassermann test. A positive finding does not necessarily exclude the man, but it becomes a matter of record. So far as medical officers know, there are no cases of transmission of syphilis directly from soldier to soldier. Gonorrheal ophthalmia is spread in some instances by infected towels. Whether or not in case of assembling a large army some earnest attempt should be made to exclude dissolute women from the vicinity of camps is a question of importance. A large Committee has charge of this matter, and suggestions will be gladly received from any source. It will certainly be a great misfortune if we assemble in camps the flower of the nation and have these young men contaminated with venereal disease.

15. *Recruits*.—In assembling a large army rather suddenly, much will depend upon those who examine recruits. Such examinations should be both rapid and accurate, and the result will depend upon the training of the officer who does it. With proper training, men can be examined both rapidly and thoroughly. Without proper training, no amount of time will give good results. This is a matter of great importance. We understand that Canadian medical officers enlisted many thousands who, after reaching England, were found to be absolutely unfit, and had to be returned to Canada. The unfit man is not only no good as a soldier, but is a detriment and a hindrance in every way. He must be fed, clothed,

and cared for, and he becomes a burden for the present, and a pensioner in the future.

16. *Manufacture of Salvarsan, and Other Articles Now Under German Patent.*—This is a chemical rather than a medical question. Salvarsan is made by the Massachusetts State Board of Health and has been made by Shamburg, of Philadelphia. The question of infringement of patent is a legal one, and will have to be taken care of by the Legal Department of the Government. Attention has been called to the necessity of making cocaine substitutes and chemists in different parts of the country have been called upon to prepare for this work. The Government should import as soon as possible such quantities of opium and cinchona as may be needed.

17. *Medical Plants.*—This subject properly belongs to the Committee on Botany of which Professor Coulter of the University of Chicago is Chairman. There are certain plants of great medicinal value, such as belladonna and digitalis for which we depend largely upon importation. These plants have been grown more or less in this country by the Bureau of Plant Industry in Washington, by the Universities of Wisconsin, Minnesota, and Oregon, and by some commercial houses. The National Research Council would like information along this line.

18. *Vermin.*—Lice infected many of the soldiers on the Texas border. Of course, these lice did not carry the typhus virus, and consequently did not disseminate typhus fever. Much has been done along this line in Europe, and Strong, who conducted the crusade against typhus fever in Serbia, will be depended upon for leadership in combating typhus fever and other diseases disseminated in the same way. A cresol preparation has been found to be most effective.

19. *Malaria.*—Some years ago Major Craig of the Army Medical Corps wrote a monograph on the Prophylaxis of Malaria as practiced by the army medical officer. The National Research Council will request that this monograph—possibly revised and brought up to date—be reprinted in sufficient numbers to be placed in the hands of every medical officer in the Reserve Corps. Certain sections of our country will require from medical officers a knowledge of the preventive measures for combating malaria.

20. *Flies.*—Experience on the Mexican border has shown that the fly problem has not been entirely and satisfactorily solved. One trouble lies in the fact that the area immediately around the military camp, and beyond military jurisdiction, is often in a bad sanitary condition. Flies breed in these localities, and spread to the camps. It has been suggested that in case large troops are assembled, the Public Health Service should be put in control of the sanitation of the surrounding country. The patrol of this service should be as extensive and as thorough as the necessities of the case demand. Possibly the Public Health Service might banish prostitutes as well as other pests from the surrounding country.

21. *First-Aid Dressing Stations.*—The object of the Council has been to learn what has been done along this line in Europe, and, if possible, suggest improvements. The first-aid rendered a wounded man influences greatly not only his

subsequent condition so far as pain is concerned, but often has a determining effect upon the final result.

22. *Motor Laboratories*.—Motor laboratories are employed in France. We intend to copy these, and to improve upon them if possible. It is desirable to learn as accurately as possible what these laboratories should carry.

23. *The Disinfection of Ambulances, Cars, and Other Vehicles Used in the Transportation of the Wounded*.—These vehicles become soiled with dirt, blood, and fecal matter. What are the best methods of cleaning and disinfecting them?

24. *Protection of the Ear From the Noises of Battle*.—Much work has been done upon injuries to the ear caused by explosions. There is considerable literature concerning this subject. An American physician, Wilson, has given special attention to this subject, and it is hoped that he will advise the Council in this and in any other matters with which his experience abroad may have acquainted him.

25. *Tuberculosis in War*.—How closely should the recruit be-examined for the discovery of early tuberculosis? Experience in France seems to show that certain cases of tuberculosis have been benefited by army service, while others have been injured. Life in the trenches has developed latent tuberculosis, while army life in the open has improved many cases of this disease, and, indeed, apparently cured them. At present it seems wise that in the examination of recruits the closest attention should be given to incipient tuberculosis; that this should not necessarily exclude a man from service, but be of value in determining the line of service to which he is assigned. Then there is the big question in France now of what is to be done with the soldier who develops tuberculosis. Shall he be sent to a sanitarium, or an open air camp, or shall he be sent home? If he has open tuberculosis, shall he be kept under supervision and so guarded that he will not scatter the infection, or shall he be allowed all of the freedom of civil life? These are big and important questions.

26. *The Psychoses of the Soldier*.—To what extent should mental tests be employed in recruiting? Should men who evidently are of unstable organization be received into the service, and if so, into what line of service may they be admitted? The Council hopes for advice from our specialists in nervous diseases on this matter.

27. *Hastening the Healing of Wounds*.—What can be done along this line? The Council will look to Carrel and other experts for advice. Robertson of the University of California has extracted from the anterior lobe of the pituitary body a substance which he calls tethelin. This substance has a pronounced effect upon the growth of animals, and it has been suggested that it might be of value in hastening the healing of wounds.

These problems have been taken up by the National Research Council. The Council will gladly receive suggestions upon these and any other scientific problems which may appear to anyone. Suggestions, help, or information of any kind will be gladly received by the Medical Committee of the National Research Council. Address—Room 908 Munsey Building, Washington, D. C.

—V. C. V.

*Are We to Forget the Lessons of 1898?*

THIS is a question now being asked by medical men all over this country. We have no desire to recall the sad story of typhoid fever epidemic among our soldiers in 1898, unless we may profit by doing so. It may be well for us to remember that out of a total of 200,000 enlisted men in that war, more than 20,000 developed this disease. But, says one, vaccination now prevents altogether, or greatly lessens the chances of developing typhoid fever, and such an epidemic can never again occur. This may be true, and is undoubtedly partly true, at least of typhoid fever, but there are other diseases for which, unfortunately, we have no protective vaccination. Some of these diseases are diarrhea, dysentery, both bacillary and amebic, scarlet fever, measles, various forms of meningitis, poliomyelitis, pneumonia, tuberculosis, etc. All infections have not yet been conquered. Among the causes of the fearful diseases of 1898—and the causes were many—was the lack of authority on the part of the medical officer. There is abundant evidence of this in various government documents. In the "Report on Typhoid Fever in United States Military Camps in 1898" the evidence that the superior authority of the line officer was responsible in many instances for the unsanitary condition of the camps is abundant. This report tells us that many commands were unwisely located, and often this was done in the face of remonstrances on the part of the medical officer. Medical officers of the Seventh Army Corps generally condemned the location at Miami, and yet regiments were kept on this site until they were much reduced by illness, and at Chickamauga some regiments were placed on ground so rocky that the construction of latrines of proper depth and width was impossible. Other camp sites received the surface washings from adjacent commands. Some were contracted into half the regulation space. Many regiments were compelled to remain on the same site until the soil became badly polluted, notwithstanding the fact that there were many broad acres around about, and no hostile army was nearer than Havana. From this report we make the following quotation: "There were regiments at Chickamauga that did not move a tenth of an inch from the time of arrival in May to that of departure late in August. Requests for change in location made by medical officers were not always granted. As an illustration under this head we may call attention to the official records of the Fifth Pennsylvania. This command reached Chickamauga Park May 20, and was, unfortunately, located on low ground. Requests for a change in location were repeatedly sent in during June and July. The soil became muddy; the camp received the washings from camps above; the sinks rapidly filled with water and overflowed, and still requests for change in location were not heeded until August 12. As we have seen, some of the regiments were improperly located from a sanitary standpoint. This was done by superior line officers, and sometimes in the face of protests from the medical officers."

This report advised that greater authority be given medical officers in all questions relating to the hygiene and sanitation of camps, and now, as we are going into another war, the line has one general officer for every 167 commissioned officers, while the medical corps has but one, whatever the number of commis-

sioned officers may be, and in an army of one million, the number of commissioned medical officers will be not less than seven thousand.

The medical profession requests that one-half of one per cent of commissioned officers in its corps have the rank of general officers. This seems a modest request, and is allowed in the navy; but, for some unknown reason, has so far been denied the army. One who has served in the medical corps can understand and thoroughly appreciate the hesitancy with which a lieutenant in that corps may recommend to a Colonel of the line that a camp site be changed, or that some other sanitary improvement is desirable; and one who served in the medical corps in 1898 knows full well the reception such a recommendation frequently met at that time, and he can guess at the reception it is likely to receive in the future under similar conditions. If any one has doubt concerning the attitude of many line officers of high rank in 1898 towards the recommendations of medical officers, he should read the testimony of Major General Brooke and other officers in command in the camps in 1898. This testimony may be found in the volumes of the Congressional Inquiry into the "Conduct of the War Department in the War with Spain," generally known as the Dodge report.

The Commanding Line Officer at Chickamauga took no pains in his testimony to show his contempt for the advice of his own medical officers, and this contempt and disregard constituted large factors in filling the hospitals and graves with typhoid cases. We have not place here to quote largely from this testimony, but a few sentences of the testimony of General Brooke may be given:

"Q.—Had any of the wells, General, been condemned by the Medical Department prior to your leaving (Chickamauga)?"

"A.—By alleged Medical Departments. Two of them were erroneous, I believe, after investigation made by myself. The one in front of a South Carolina Regiment which, I believe really to have been perfectly pure water. There was afterwards discovered a surface well which had been walled up and water slipped in from which this Regiment—this was reported to me—used the water. That well was not far from a large sink, and possibly on lower ground. I never could understand from my knowledge of rocky strata how that well could have been contaminated. Another well lying on the road between Alexander House and Jays' Mill was also condemned. I drank of that well water every time I passed it until somebody broke the pump to pieces. I suppose it was some of our energetic medical fraternity who had spent their time in finding that there was a suspicion of these two wells—and then I did not bother further about it."\*

On the following page there is a statement of the unsanitary condition of Camp Thomas made by the medical officer of the Twelfth New York Infantry. The commanding Line Officer was so incensed at this report that he said: "If you will give me a copy of that report, I will see that that young man goes before a court-martial for the sort of statement he has made there if he is not protected by this commission."

On July 17, 1898, the Chief Medical Officer at Chickamauga addressed a letter to the Adjutant General containing recommendations concerning the improvements of sanitary conditions. In brief the letter contained the following recommendations:

\*Conduct of War Department in War with Spain, vi, 3080.



1. That the signal corps which had occupied the same site for several months, and which was crowded, should be moved.
2. That selected places should be designated as dumping grounds, and all the waste should be collected and deposited on these places instead of being scattered through the camp.
3. That so far as possible all camp sites should be changed.
4. That the hospital of the First Division of the First Corps be moved from the unsanitary position they occupied to a more healthful location.
5. That the village of Lytle, which was a sanitary menace to the troops, should be cleansed.
6. That all condemned sources of water supply should be effectually closed.
7. That only filtered or boiled water should be used by the soldiers.
8. That all hucksters selling doubtful food or drink should be expelled from the camp.
9. That there should be careful supervision of all food and drink sold in the canteens.

These recommendations made in July were unheeded at the time.

In his testimony, General Brooke spoke of the letter containing the above recommendations as follows: "I did not regard his letter in a very serious sense. I do not know how he came to write it. There was much complaint in that camp from men of his own profession as to his action. He caused me more trouble and annoyance than anyone ever did."

Had the recommendations contained in this letter which annoyed the Senior Line Officer been taken seriously in July, the fearful harvest of sickness and death in August might have been averted.

Nineteen years have passed since our little war with Spain, and we have crossed the threshold of a great war with Germany, Austria, Bulgaria and Turkey. This war begins with the medical officer possessed of no more authority than he had in 1898. Will his recommendations be as futile as they were then? The medical profession has always been responsive to its country's demands, whether in war, in pestilence, in flood, or in famine. Conscription has never been necessary to fill its quota. Medical officers will do their best and will present their recommendations to superior line officers, but they realize that these recommendations are likely to receive scant attention, and that the medical officer will be compelled to work under a heavy handicap. The government stamp placed upon the medical officer indicates the opinion that the government has of the value of his services, and that his recommendations will receive from line officers any different consideration from that accorded them in 1898 is not probable. At present the Army Medical Corps has no representation on the General Staff or in the War College.

Will it be possible that camp sites, both small and great, will be selected as they were in 1898 without consultation with the medical corps? And are we justified in feeling that we may have some reminders of the experiences of 1898? According to the testimony of the Surgeon General recently, given before a medical committee, the relative number of trained medical officers is not as great now as it was at the beginning of the Spanish War. We had then seven per thousand. We have now about five per thousand.

—I. C. I.

*Ambard's Coefficient*

THE methods which have been developed in recent years for the analysis of blood in normal or pathological conditions have opened many fields of investigation. Perhaps the most important of these, from the standpoint of medicine, is the problem of renal function. Not long ago, the clinician had to be content with a very elementary analysis of the urine in cases where the kidneys were suspected of being diseased. The methods for the analysis of the blood for the urinary constituents were not suitable for use in the clinic, and the simple procedure of drawing blood from a vein was thought to be a rather serious measure, because of fear of introducing bacteria into the circulation. Thanks to the development of first-class chemical methods for the quantitative determination of the urinary nitrogenous bodies in small quantities of blood, and the general use of venous puncture brought about chiefly through the development of the Wassermann reaction, our knowledge of the intermediary metabolism of the proteins in disease has been greatly extended. As it is now possible to determine with ease the quantity of the urinary constituents in both blood and urine, it is not surprising that numerous researches have been directed to show the relationship which exists between them.

Our knowledge of the kidney is still in an incomplete state. There are two schools of thought in regard to the fundamental process which is responsible for the excretion of urine, i. e., the physical and the vital schools. Perhaps the majority of physiologists believe that both physical and vital processes play a role. Certainly there are many phenomena in the excretion of the urine which cannot be adequately explained at present either on a purely mechanical or on a vital basis. Nevertheless, if physical processes play the most important role in the secretion of the urine, it should be possible to formulate fairly definite laws which will mathematically express the relationship of the concentration of the urinary bodies in the blood and the urine, and the rate of their excretion by the kidney. In the urine we find a concentrated solution of nitrogenous waste material; in the blood the reverse is true. For years it has been thought that there is no definite relationship between the concentration of urea in the blood and the rate at which the kidney secretes the urea. Undoubtedly the blood flow and the blood pressure in the kidney are important factors in this connection, but they cannot be experimentally determined.

About six years ago two Frenchmen, Ambard and Weill,<sup>1</sup> announced some laws which reduce the study of renal function to physical laws. They believe that the urea in the blood acts as a stimulus to the renal cells, and that the rate at which the urea is secreted varies with the strength of this stimulus. In support of this postulate they collected a number of cases in which the concentration of the urea in the urine was alike, and they found that the rate of the excretion of urea in these cases varied directly as the square of the concentration of urea in the blood. This may be mathematically expressed in the form of the simple proportion:

$$\frac{(\text{Grams of urea per 100 c.c. of blood in case A})^2}{\text{Grams of urea excreted in the urine per hour in case A}} = \frac{(\text{Grams of urea per 100 c.c. of blood in case B})^2}{\text{Grams of urea excreted in the urine per hour in case B}}$$

According to Ambard and Weill, the rate of the circulation of the blood through the kidney is the factor which governs the concentration of the urine. A dilute urine is a sign of a fast circulation, a concentrated one of a slow circulation. In connection with this, they found a number of cases in which the concentration of the urea in the blood was alike, and in these cases the rate at which the urea was excreted by the kidney varied inversely as the square root of the concentration of the urea in the urine. This may be mathematically expressed in the form of a proportion:

$$\frac{\text{Rate of excretion of urea in the urine in case A}}{\text{Rate of excretion of urea in the urine in B}} = \frac{\sqrt{\text{The concentration of the urea in the urine of case B}}}{\sqrt{\text{The concentration of the urea in the urine of case A}}}$$

In other words, the greater the concentration of the urea in the urine, the blood urea remaining constant, the slower the rate of elimination of the urea. By combining the two laws they obtain an equation which gives a constant, this constant expressing the relationship between the rate of excretion of the urea in the blood and in the urine. In order to make this constant standard in all cases, they adopted a standard body weight of 70 kilos and a standard concentration of the urine of 25 grams per liter. These corrections they include in the formula, which reads as follows:

$$K = \frac{Ur}{D \times \frac{70}{P} \times \frac{\sqrt{C}}{\sqrt{25}}}, \text{ in which:}$$

K = coefficient of urea excretion (Constant of Ambard).

Ur = grams of urea per liter of blood.

D = output of urea in grams per 24 hours.

P = weight of the patient.

C = grams of urea per liter of urine.

70 = standard weight.

25 = standard concentration of the urine.

The average value for this constant in normal individuals they found to lie between .06 and .09. In cases of renal disease the coefficient is usually raised. Maclean and Sellings were the first in this country to use the coefficient of Ambard as a test of renal function. By using methods for analysis superior to those used by Ambard, they found that the coefficient of Ambard is a little low. They also propose a modification of the formula of Ambard which in no way changes the principles involved, but expresses the normal value in terms of 100. Their formula is

$$\frac{\text{Grams urea per 24 hours excreted in urine}}{\text{Weight of body in Kg.}} \times \sqrt{\frac{\text{grams urea per liter excreted in urine}}{(\text{Grams urea per liter of blood})^2}} \times 8.96 = \text{Index of excretion.}$$

In the above formula the standard weight and standard concentration of the urea employed by Ambard are present in the factor of 8.96.

Using this formula Maclean<sup>2</sup> computed the relation of the rate of urea excretion to the concentration in the blood in 107 observations on individuals with normal excretion. From these data he concludes that the rate of the excretion of urea can be measured directly in terms of the normal by the use of the modified

Ambard's coefficient. The normal average concentration of urea in the blood in the above cases varied between about .2 and .5 grams per liter in the same or different individuals, and the rate of excretion appeared to be controlled by this concentration and by the rate of water excretion. Below values of .3 grams per liter the laws do not hold so closely, since there is a tendency in such cases towards a higher rate of excretion. He calls particular attention to the larger number of cases (71) in which the blood urea is between .3 and .5 grams per liter since it is within these limits the blood urea figures may be normal or signify retention. Of these, 52 gave an index within ten per cent of the range of normal values, which may be considered, he believes, to lie between 80 and 125; 69 of these, i. e., 97 per cent, come within a range of 25 per cent of the normal value. Of the 107 cases reported only three had an index below 80, which he believes indicates impairment of renal function.

As a means of testing the validity of Ambard's laws, Maclean calculated in each case, in addition to the index of urea excretion, the following formula:

$$\frac{\sqrt{\text{Urea excreted in 24 hours}} \times \sqrt{\text{Concentration of urea in the urine}}}{\text{Weight}}$$

This formula expresses Ambard's laws in the simplest form, and it is not distorted by the addition of constants used in the coefficient. There is quite a variation in the index he obtained by this formula, and this is true even where one would expect the laws particularly to hold, as in cases where the concentration of urea in the blood was the same. The most marked variation is found in the cases where the blood urea is below .3 grams per kilo, in which there are variations of 33 per cent of the value of the normal figure, which Maclean fixes at 30 as this corresponds to 100 in the complete formula. In this form it is apparent that, by the use of the square of the urea in the blood as a part of the denominator, a somewhat wide variation in the value of the constant or percentage of normal excretion will be obtained, just as is the case with the constant when we use the square of the urea of the plasma in expressing Ambard's first law. The normal limits of variation once established, the modified index of Maclean is better, since it expresses the urinary urea excretion in terms of the per cent of normal. With the higher concentrations of urea the coefficient is sufficiently constant, Maclean believes, to be of use in testing renal function by the use of the complete formula proposed.

Recently Addis and Watanabe have published a good series of observations on normal individuals, in which the two separate laws on which Ambard bases his coefficient are more thoroughly tested than by either Ambard or Maclean.<sup>3</sup> They have arranged their cases in two classes: one in which the cases are classified in groups having the same concentration of urinary urea, and another in which the groups are made of those having the same concentration of blood urea. They hold that if Ambard's postulates are true, each instance within the different groups must possess the same constant when the laws are applied. They conclude from their results that, since the variations found are entirely too great to be due to experimental error, factors other than the concentration of the blood urea must commonly intervene in the process of urea excretion.

Addis and Watanabe rightly call attention to our lack of complete knowledge

of the factors governing the excretion of urea by the kidneys. They feel that the impression that these factors are known and measurable with mathematical accuracy would be likely to retard further investigation. The constancy of the combined formula, which after all appears to be only roughly approximate, is due in large part to the mathematical construction, and also to the fact that any increase in the concentration of urea in the blood is usually accompanied by an increased rate of urea excretion. The factors which are most variable occur as the square or the fourth roots of their values, and thus the disturbing effect they produce on the constancy of the resultant of the formula is greatly reduced, while the most constant factor, the concentration of urea in the blood, is used without modification. As these critics suggest, in such a complex mechanism it is very probable that other factors are of great importance in controlling the rate of urinary excretion. Many of these factors cannot admit of mathematical expression.

It may be that Ambard's laws roughly express in mathematical form one of the important factors in urinary excretion. In the healthy normal kidney, however, there are vital factors which even Ludwig, who first formulated the mechanical theory of urinary excretion, was forced to accept. It may be that the wide variations which all careful workers find present in the coefficient in normal individuals is simply an indication that the physico-chemical factors are not relatively so important in the normal kidneys as they are when the vitality of the kidney is decreased.

In pathological conditions in which the kidney's excretory power is diminished, we should naturally expect an increase in the concentration of the urea in the blood, or a decrease in the concentration of the urea in the urine and an increase in blood pressure, if physical laws are to compensate for the loss of the vital activity. Most of these factors are present in chronic nephritis. In this connection the work of Mosenthal<sup>4</sup> is most illuminating. He finds that the diseased kidney can no longer concentrate the urine. Is it not significant that the ability of the kidney to concentrate the urine was the point which Ludwig could not explain upon a mechanical basis?

The French have used the coefficient for some time as a measure of renal activity. Maclean has reported a series of observations on patients suffering with renal disease. Very recently Lewis<sup>5</sup> has published an extensive report in which he discusses the clinical value of the coefficient. He finds, as the previous observers have done, that the normal variations are rather wide and are subject to many disturbing influences. In hospital practice he has observed, however, that the variations in the normal figure are small, and that in cases where the metabolism is much increased, as in fevers, in exophthalmic goiter, in hypertension with early changes in the renal arterioles, and in early chronic diffuse nephritis, there is a depression of the coefficient of Ambard. This depression he believes indicates an increase in renal activity. He finds that the coefficient is raised in cases where there is evidence of decreased metabolic activity, as in myxedema. Similarly he finds that, in myocardial insufficiency and in nephritis with renal insufficiency, the coefficient is raised. This appears to indicate that, when the vital activity of the kidney is in any way disturbed, the physico-chemical factors at once play a relatively greater part in the renal excretion. He finds a

marked uniformity in the coefficient and the results obtained by the phenolsulphonephthalein test in all stages of nephritis, and believes that the test has considerable prognostic value.

Lewis takes issue with Addis and Watanabe on the point that the concentration of the blood urea is really the factor which determines the constancy of the coefficient, for he cites cases in which the blood urea was markedly reduced by dietary measures without any effect on the value of the coefficient.

This problem is very interesting, but there are many points which must be subjected to most severe scrutiny before the coefficient is accepted as really being anything more than a rough indication of what the vital activity of the renal cells is, or as being superior in any way to the phenolsulphonephthalein test, or to the more physiological test advocated by Mosenthal. With the use of the newly developed methods of blood and urine analysis for urea, we hope that future work will be able to differentiate clearly between the vital factors of renal activity and the factors which the laws of Ambard attempt to express.

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<sup>2</sup>Maclean: *Jour. Exper. Med.*, 1915, xxii, 213, 366.

<sup>3</sup>Addis and Watanabe: *Jour. Biol. Chem.*, 1916, xxiv, 203.

<sup>4</sup>Mosenthal: *Arch. Int. Med.*, 1915, xvi, 733.

<sup>5</sup>Lewis: *Arch. Int. Med.*, 1916, xix, 1.

—R. G. P.

### *Pulmonary Osteoarthropathy*

**H**YPERTROPHIC pulmonary osteoarthropathy is an interesting condition not because of its effect upon the life of a patient but because of its associated organic diseases. Described first by Marie (1890), and shortly afterward by Bamberger, it has become one of the many interesting anomalous bone diseases the causes of which are not known. It is of clinical interest because of the resemblance it gives, in the hands particularly, to acromegaly; and because of the hint it gives of thoracic, and especially pulmonary, trouble. It has been described as frequently associated with chronic pulmonary tuberculosis, with bronchiectasis, with pleural empyema, and with mediastinal tumors. Nevertheless, a recent textbook on tuberculosis states that in 2,300 tuberculous patients not a single case of osteoarthropathy was observed.

Recently, that is to say within the past ten years or so, several interesting reports have come from Locke<sup>1</sup> and Brooks.<sup>2</sup> These are summarized, in the latest account of the disease, by Kessel.<sup>3</sup>

Kessel has made radiographic studies of the hands, feet, all of the long bones, the bones of the pelvis, and in some instances the shoulder girdle and cranial bones, with the interesting result that he has found evidences of hyperplastic growths connected with the bones in 32 cases, and has found evidence, in addition to that given by Janeway<sup>4</sup> and Locke, that simple clubbing of the

<sup>1</sup>Locke: *Arch. Int. Med.*, 1915, xv, 659.

<sup>2</sup>Brooks: *New York Med. Jour.*, Sept. 27, and Oct. 4, 1913.

<sup>3</sup>Kessel: *Arch. Int. Med.*, 1917, xix, 239.

<sup>4</sup>Janeway: *Am. Jour. Med. Sc.*, October, 1903.

fingers and hypertrophic osteoarthropathy are stages of the same process, and not different processes as certain French and German authors believe.

The etiologic factors are only guessed. It is usually conceded, says Kessel, that prolonged venous congestion is the cause of the ordinary clubbed fingers, and Brooks, following Ziegler, believes that a continued peripheral hyperemia is produced by compression of the lung capillaries in pulmonary, pleural, and mediastinal disease, and this in the course of time leads to hyperplasia of the periosteum and connective tissue. In addition to this hyperemia a circulating toxin probably plays a role, say the writers. This it may be said is all guess work.

However the changes are produced, they consist in a progressive ossifying periostitis, usually manifesting itself in the distal ends of the diaphyses of the long bones and later involving the other bones of the skeleton. The earlier stages, or the milder forms of the disorder, appear to be mere hypertrophy of the soft tissues with thickening of the nails. Many patients also show a globular nose and evidence of thickening of the subcutaneous tissue in the malar regions.

Kessel's cases arrange themselves in three groups. In the first are five patients with simple clubbing of the fingers without bone changes. In the second are seventeen patients with clubbing of the fingers and bone changes. In the third are ten patients with clubbed fingers and changes in the long bones. The bone changes do not run parallel, in severity, with the tuberculous process.

—P. G. H.

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### *The Rank and Authority of the Medical Officer*

WHEN Stanton was Secretary of War, and Hammond, Surgeon General, early in the Civil War, the latter made a request of the former for advanced rank for medical officers. The great Secretary of War replied with a question: "Will increased rank make your medical men better doctors?" The Surgeon General replied with another question: "Does increased rank make Line Officers, Quartermasters, and those in other corps more proficient?"

There are two important considerations in regard to the rank and authority of army medical officers. In the first place, the higher the rank obtainable, the better the class of young physicians attracted to the corps. When a young man knows that whatever he may do, however skillful and energetic he may be, whatever discoveries he may make, whatever sacrifices he may undergo, the rank of Colonel, with a pay of about \$5,000 is the best that he can possibly look forward to in his old age, it must be acknowledged that the temptation to enter the army medical service is not great.

In the second place, and this is of more importance, rank in the Army necessarily means much. A request or a recommendation from a Colonel or a General will receive more consideration than when it comes from a Lieutenant. Much of the disgrace of 1898 and the disregard shown their recommendations by superior line officers, was due to the lack of rank and authority among medical men.

P. C. F.

## *The English Recognize the Importance of Giving Authority to Medical Officers*

IN 1904 the English War Office was reorganized by a committee, the chairman of which was Lord Esher. In this reorganization, no provision was made for a representative of the Medical Army Corps on the General Staff, or what corresponds to our War College. At the time the Surgeon General complained of this action. In reply to this complaint Lord Esher's committee stated that while too much importance could not be attached to the sanitary service of the army in peace or in war, the committee could not accept the views of the Surgeon General. Lord Esher's committee continued, "The Army Council is not, and can not be, a representative body as regards the several arms and departments. The Royal Army Medical Corps exists to serve the Army in a most important capacity, but the first object must be to create and maintain an Army, and this is the function of the Army Council. To admit the principle of representation would destroy the character of the Council."

This was the opinion of Lord Esher in 1904. Recently (*London Times*, February 3, 1917) Lord Esher writes as follows: "How much of the suffering undergone by our soldiers since the war began has been due to the shortsightedness of my committee, and notably of myself, will never be known. Certainly the control of the Adjutant General's branch over the Royal Army Medical Corps, was and is responsible not only for the early failure to grip the medical factors of the war, but they hampered conditions under which the Surgeon General has worked. His triumphs, and those of the Royal Army Medical Corps have been achieved in spite of obstacles that the subordination of science to ignorance, and of elasticity to military discipline explains, but can not justify."

—F. C. V.

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## *Intestinal Obstruction*

THE cause of the rapidly developing and quickly fatal toxemia which follows acute intestinal obstruction has never been satisfactorily explained. Numerous theories have been formulated, many of which are based upon experimental data, but they differ widely. Clinical and experimental evidence fails to show the presence of a bacteriemia in many cases. In the normal bowel, substances are found which are toxic when injected into the blood stream, and experimental evidence indicates that the substances obtained from the obstructed gut are more toxic than those from a normal one, but the relationship of these toxic substances in the intestines to the symptoms and the factors involved in the production and the absorption of the toxic materials are unsettled. Recently several papers have appeared which throw some light upon the question.

Whipple, Cook, and Stearns report experiments in which they have injected a protein substance (proteose) obtained from the contents of isolated closed loops of intestines in dogs and from those of the intestines of clinical cases of intestinal obstruction. They find that there is a definite increase in the non-protein nitrogen of the blood following the injection of the proteose substance.



Likewise they find an abnormally high nonprotein content in the blood of cases suffering with intestinal obstruction and in dogs with closed intestinal loops. The proteose intoxication is accompanied by the usual symptoms of obstruction; viz., vomiting, diarrhea, low blood pressure and a temperature reaction. They believe that the proteose produces a tissue reaction, which disturbs the delicate equilibrium of the numerous physicochemical reactions in the tissue cells, and that the results of their experiments indicate that the symptoms of intestinal obstruction are produced by the absorbed proteose in the obstructed bowel. They have been able to show that repeated injections of sublethal doses of the proteose material confer upon an animal a relative immunity to its toxic action. Dogs in which such an immunity has been established survive a closed loop of intestine in the abdominal cavity, or an intestinal obstruction, longer than animals which are not immune. The immune animal can also withstand the action of a lethal dose of proteose substance. An examination of the urine in these cases shows that during the intoxication and for some time following the reaction, there is an increased excretion of nitrogen above the fasting level, and that this increase is, in general, proportional to the severity of the reaction. They believe that the increase in nitrogen excretion indicates a general breaking down of tissue proteins and that this plays an important role in the intoxication.

In the same number of the journal in which the above mentioned experiments appear, Dragstedt, Moorhead and Burcky, working in Carlson's laboratory, report some very interesting and important experiments which contribute to the support of the proteose theory of intoxication in obstruction. A large number of observations were made on dogs in which loops of intestines were isolated and the integrity of the bowel restored by joining the free ends. In all the cases in which the loop was closed, the animal died with the symptoms of acute intoxication or peritonitis. Loops made high up in the bowel were more rapidly fatal than those made lower down. In one series of cases the loops were thoroughly washed out with water and ether before they were closed. A fair percentage of the animals recovered and lived normally. Examination of the loops showed that bacteria were present in the contents. Sudden obstruction of the blood supply of these loops resulted in acute symptoms of intoxication and obstruction, and then death. In a number of dogs, loops of intestines were isolated and after being thoroughly washed were replaced in the abdominal cavity without being closed. Some of these animals lived. Later examination showed that the ends of the loops had closed and that the contents were sterile. Occlusion of the blood supply to these loops caused no symptoms. In another series, loops from the duodenum and the jejunum, having open ends and without being washed, were dropped back into the abdominal cavity. Three dogs, or one-half the number in the series, recovered. Thirty days later, examination showed the loops closed and distended with fluid.

The experiments are very instructive, since they demonstrate that the mere presence of bacteria alone in the loop, is not sufficient to cause symptoms, and that the secretion of the bowel is not the toxic agent. They indicate that besides bacteria, there must be present in the bowel toxic products resulting from the action of putrefactive bacteria on necrotic tissue to give symptoms of obstruc-

tion. Any interference with the blood supply when bacteria are present produces tissue necrosis. This condition simulates acute obstruction in man, in which there is an accompanying occlusion of the blood supply to the part (volvulus, strangulated hernia, etc.). The experiments seem to show that the toxemia which is produced by a closed intestinal loop without interference with the blood supply, is not similar to that found in intestinal stasis.

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—R. G. P.

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## ORIGINAL ARTICLES

### SMALLPOX VACCINATION AT THE UNIVERSITY OF CALIFORNIA\*

BY J. N. FORCE, M.D., BERKELEY, CALIF.

THE Regents of the University of California require that all intrants shall show satisfactory vaccination scars or other evidence of immunity against smallpox.

In determining what constitutes a satisfactory scar we have not been influenced by the size or character, since complete immunity to smallpox vaccine may exist in an unscarred person or in one whose scar is entirely free from characteristic pits. The duration of immunity, however, varies inversely as the age of the vaccination scar. In a series of 318 persons showing vaccination scars under ten years old, 48 per cent were completely immune as indicated by the cutaneous allergic reaction of immunity, 38 per cent were partially immune as indicated by the secondary vaccinia (vaccinoid), and 14 per cent showed no immunity to revaccination as indicated by the primary vaccinia produced. In a similar series of 444 persons whose scars were between ten and twenty years old, the same percentages were obtained. In a series of 136 persons whose scars were over twenty years old, 38 per cent were completely immune, 27 per cent were partially immune, and 35 per cent showed no immunity to revaccination. Since we have reason to believe that immunity to smallpox is more lasting than immunity to vaccination, we ignore the 14 per cent of nonimmunes and set the limit for revaccination at ten years.

If an intrant has a scar over ten years old he is vaccinated in two spots on the arm, a control spot being scarified but not vaccinated. Observations are made at twenty-four hour and five day periods after vaccination. If either of the vaccinated spots show an areola of 5.0 mm. or over (with or without papule)

\*Submitted to the National Research Council by Dr. E. P. Gay, University of California, Member of Committee on Medicine and Hygiene.

at the end of twenty-four hours, which areola (or papule) has decreased at the time of the fifth day observation, it is considered a *reaction of immunity* due to the presence of antibodies against smallpox vaccine in the tissues and the sub-



Fig. 1.—Reaction of immunity twenty-four hours after vaccination. The upper spot is a control. There are 5 mm. areolæ around both vaccinated spots.



Fig. 2.—Reaction of immunity forty-eight hours after vaccination. The areolæ have increased slightly and there is papule formation.



Fig. 3.—Vaccinoid sixth day after vaccination. The reaction has attained its maximum. The previous scar is twenty years old.

ject is excused from further vaccination. If either of the vaccinated spots show an areola at the end of twenty-four hours which develops into a small vesicle which matures on the fifth or sixth day and then rapidly subsides, the reaction is con-



Fig. 4.—Vaccinia sixth day. Compare with Fig. 3.



Fig. 5.—Vaccinia eleventh day. The height of the reaction.



Fig. 6.—Scars resulting from the vaccination produced by three scarifications 2 mm. in diameter. The scars measure 11 mm. in diameter each.

sidered a *secondary vaccinia* (*vaccinoid*). If there is no change until the third day, and then a small areola begins to form, the case will be one of *vaccinia*. If the small areola has not become a vesicle by the fifth day the smallpox vaccine is probably incapable of producing vaccinia, or was not properly brought in contact with the derma. The vaccination is accordingly repeated with fresh vaccine. If the subject is not seen until the fifth day and there are then no signs of a recent local reaction, the vaccination is repeated, for all signs of the reaction of immunity may be gone by the fifth day following vaccination. Inert vaccine is capable of producing the reaction of immunity in an immune subject.

Repeated vaccination with an inert vaccine may finally render the subject immune to potent vaccine, or a small scar may disappear though the immunity persists. In a series of 215 intrants showing no vaccination scar but giving histories of from one to twenty previous vaccinations, one per cent were immune, six per cent were partially immune, and 93 per cent were not immune to smallpox vaccine. In our experience no person who has been given internal vaccination has shown the slightest immunity to smallpox vaccine. In a series of unscarred intrants with a history of smallpox, 36 per cent were completely immune, 40 per cent were partially immune, and 24 per cent showed no immunity to smallpox vaccine. We have never produced a primary vaccinia in a person who showed the characteristic pits of smallpox, but in many instances we have produced the secondary vaccinia (*vaccinoid*) in such subjects.

In a series of 465 unscarred intrants, claiming never to have been previously vaccinated, *not one person was completely immune*, 1.3 per cent showed a secondary vaccinia indicating partial immunity, and 98.7 per cent showed no evidence of immunity to smallpox vaccination.

This extremely satisfactory record of 100 per cent of vesicle formation in previously unvaccinated intrants, and 99 per cent of vesicle formation in previously vaccinated intrants (unscarred) we consider due to care of the vaccine and uniform vaccination technic.

#### CARE OF THE VACCINE.

In a series of experiments performed with smallpox vaccine of various ages and different methods of preparation the following conclusions were reached:

Vaccines shipped in a vacuum bottle from the New York City Health Department gave 100 per cent vesicle formation in the previously unvaccinated. Vaccines prepared according to the method of Noguchi showed rapid deterioration giving 4 per cent of failure on second passage, 50 per cent on fifteenth passage, and 90 per cent of failure on the twenty-ninth passage of a strain received from New York. Vaccines kept constantly on the ice except when in transit from the manufacturing laboratories gave a very low percentage of failure four, five, and six months after collection, while vaccines used within a month after collection gave 100 per cent of vesicle formation in the previously unvaccinated. Since temperature is so important a factor in the potency of smallpox vaccine, precautions should be taken to avoid a long period of transit except in vacuum bottles.

A properly cooled vaccine should give absolutely 100 per cent vesicle formation in the previously unvaccinated. Since the object of vaccination is to secure immunity, successful vaccination consists in producing either a primary vaccinia, a secondary vaccinia (vaccinoid), or a reaction of immunity. A reaction of immunity in a previously unvaccinated person indicates previous smallpox. A natural immune (provided such a person exists) would give no reaction of immunity. A vaccination certificate, therefore, should be an evidence of immunity, not a confession of failure.

#### UNIFORM VACCINATION TECHNIC.

The following technic is employed in all vaccinations. A vial containing 1.0 c.c. of smallpox vaccine (which is enough for 250 vaccinations) is opened, placed in cracked ice, and covered with a bell jar. The arm in the region of the deltoid insertion is washed with alcohol and dried with sterile cotton. A small chisel provided with a carbon steel point is sterilized by dipping in alcohol and flaming. Three circular scarifications each 2.0 mm. in diameter are made through the epidermis by rotating the chisel against the tightly drawn skin. A sterile toothpick is used to transfer a drop of smallpox vaccine from the containing vial to the three scarifications, and to rub it into the exposed derma. If tube vaccine is used it is expelled on the spots and rubbed in by means of the chisel. The site of the vaccination is then covered with a square of sterile gauze held in place by adhesive tapes on one of which is stamped the return date, five days subsequent to the day of vaccination.

The vaccinia produced by this technic follows a fairly constant course. In a group of about 300 vesicles measured on the fifth day, the minimum diameter was 3.5 mm., the average fell between 6.0 and 6.5 mm., the maximum was 9.0 mm., while 70 per cent were between 5.5 and 7.5 mm. The vesicle attains its maximum development on the eleventh day with an average measurement of 12.0 mm. Between the eleventh and fifteenth days the vesicle rapidly dries and shrinks to a brown scab which, in at least 60 per cent of the cases, becomes loose enough to be easily detached by the twentieth day. The areola surrounding the vesicle closely parallels its growth until the seventh day when it increases rapidly in size from an average of 10.0 mm. to an average of 40.0 mm. This sudden increase of the areola marks the rise of antibody formation, the vaccine organisms are digested, their toxic products give rise to the constitutional symptoms of fever and headache, while the change from vesicle to pustule marks the end of the vaccine colony. The areola rapidly shrinks to the size of the scab and gives color to the fresh scar which averages 11.0 mm. in diameter. This course is followed irrespective of the preparation of vaccine used or its bacterial content. Treating with antiseptics the vaccine vesicle produced by this method of vaccination does not decrease the area of redness or shorten the course of the vaccinia.

Vaccinias of long duration and marked severity result from improper vaccination technic. Given a smallpox vaccine of proven potency, we believe that

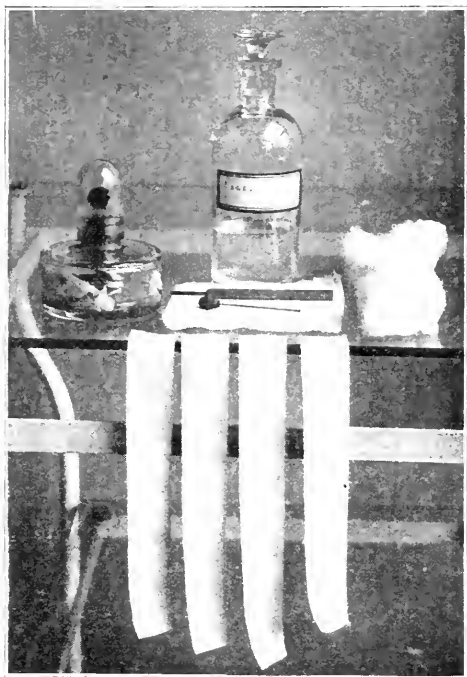


Fig. 7.—Equipment necessary for vaccination with ordinary tubed vaccine.



Fig. 8.—Cleansing the arm with alcohol and cotton.



Fig. 9.—Flaming the chisel which has been previously dipped in alcohol.





Fig. 10.—Scarification of the arm. Three circles of epidermis are removed exposing the derma.



Fig. 11.—Dropping the vaccine on the scarified spots.



Fig. 12.—Rubbing the vaccine into the scarifications.



Fig. 13.—Dressing of gauze and adhesive tape. One tape bears the return date.



Fig. 14.—Cross-scarification. One of the causes of "bad" arms.

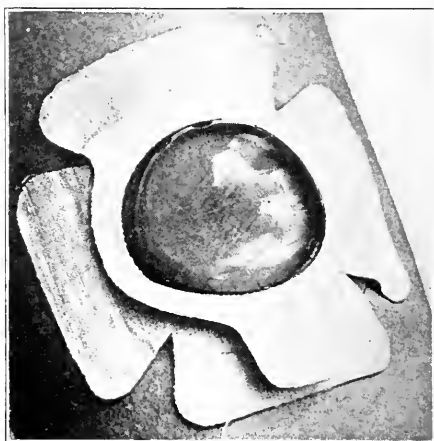


Fig. 15.—The vaccination shield. Another cause of "bad" arms.



Fig. 16.—Scar resulting from improper vaccination. Central necrosis, slough, infection, and a slowly healing ulcer. Patient thinks he is well protected because he had such a "hard take."

uniformly successful vaccinia, uncomplicated by secondary infections, may be secured by the use of small multiple scarifications. Since the vaccine colony grows only in unbroken skin, we wish to confine the growth to the smallest possible area for any one insertion. Cross-scarification leads to a crust which furnishes shelter for possible secondary invaders, the large rambling vesicle is easily broken and there is a tendency for central necrosis with a slowly healing ulcer. The results of insertion by single scratches are less constant than by the circular scarifications.

We believe that this method can only be improved by intradermal inoculation of a definite quantity of smallpox vaccine and we are working on this latter method at present.

# REPORT ON PROPHYLACTIC VACCINATION WITH *B. TYPHOSUS*, *B. PARATYPHOSUS A*, AND *B. PARATYPHOSUS B*.\*

BY WILBURT C. DAVISON, M.D., BROOKLYN, N. Y.

RECORDS of the Franco-German War, the Boer War,<sup>1</sup> the Russo-Japanese War<sup>2</sup> and the Spanish-American War<sup>3</sup> clearly show that the number of deaths from enteric fever in those wars was very large and closely approximated that due to wounds. The success of the work done by Wright, Russell,<sup>3</sup> Kabe-shima<sup>4</sup> and others in regard to immunity against typhoid fever has been admirably demonstrated by its practical application in the armies of the United States, of Japan, and of all the countries now at war. It may be safely stated that since prophylactic vaccination against typhoid fever has been made more or less universal among armies, the losses from that disease have become practically negligible (that the records of prophylactic inoculation against typhoid in the Boer War is not more favorable is due to the fact that typhoid cases were not always distinguished from paratyphoid A and B).

During the present war, the paratyphoid infections have assumed the prominent place formerly held by typhoid. Official figures are not obtainable but conservatively stated there have been several thousand cases of fever due to *B. paratyphosus A* and *B*, while only a few hundred have been due to *B. typhosus*, the majority of the latter being in unvaccinated individuals. The relative frequency of *B. paratyphosus A* and *B* varies according to the location in which the epidemic arises. In India and along the Mexican border, *B. paratyphosus A* predominates; in Gallipoli and France, *B. paratyphosus B* is the more frequent.

The recent campaign of our troops in Texas and Mexico has also clearly indicated the efficacy of prophylactic typhoid vaccination; for the American Army receives as a routine three doses of typhoid vaccine of 500 million, 1,000 million, and 1,000 million bacilli respectively, at intervals of 7 to 14 days.

The very urgent need for similar protection against the paratyphoid infections is also seen, for while the number of cases of typhoid fever at the Mexican border was practically nil, there were hundreds of cases of paratyphoid. Captain Berry<sup>5</sup> reports that 21 per cent of the Fourteenth Regiment New York Infantry developed paratyphoid fever at some time during its three months on the Mexican border at Mission, Texas. Similar facts are shown in the studies at Camp Whitman in New York. The ease with which a paratyphoid epidemic spreads is demonstrated by the fact that twelve cases of paratyphoid fever were reported in the Seventy-first New York Regiment, ten to fifteen days after their one night camp at Mission, Texas, during the hike course, and fifty cases were found among their two hundred native laborers. The need for prophylactic paratyphoid vaccination is again demonstrated by the recent examinations for the detection of carriers among the men of the Fourteenth Regiment New York Infantry after its return from Texas. Four per cent of this regiment were found by Krumwiede and Kohn<sup>6</sup> to be carriers of *B. paratyphosus A*,

\*Submitted to the National Research Council by Dr. John Howland, Medical Department Johns Hopkins University, Member of the Committee on Medicine and Hygiene.

and *B. paratyphosus* B, though there was no evidence of their having suffered from acute disease.

Prophylactic paratyphoid vaccination is practically the sole means of preventing such epidemics of this disease as occurred in France, Gallipoli, and along the Mexican border.

Castellani,<sup>7</sup> Cummins and Cumming,<sup>8</sup> Kabeshima,<sup>4</sup> Vincent, Dreyer,<sup>9</sup> Ainley Walker,<sup>10</sup> Davison,<sup>11</sup> and others have demonstrated the advantages of prophylactic vaccination with combined typhoid, paratyphoid A and paratyphoid B vaccines. Kabeshima has not only shown that simultaneous inoculation with a vaccine containing equal parts of *B. typhosus*, *B. paratyphosus* A, and *B. paratyphosus* B produced immunity against these three infections in man and animals but he also has proved that the reaction to this so-called "triple vaccine" was not more severe than that due to the typhoid vaccine alone.

One of the problems in relation to prophylactic vaccination against the enteric fevers is whether or not a better immunity against these infections is obtained by inoculating with the three microorganisms simultaneously or by immunizing with each bacillus separately by means of a succession of inoculations. It is in regard to these questions, as well as in regard to the course of immunity (as measured by agglutinins) that, at Dreyer's suggestion, I performed, in 1915 and 1916, under the supervision of Ainley Walker, a series of inoculations in a number of men and rabbits with vaccines prepared from cultures of one, two, or three of the bacilli (*B. typhosus*, *B. paratyphosus* A, and *B. paratyphosus* B), varying the doses and the order of their introduction in the successive immunizations of different groups of subjects (unpublished work). For instance, one group received the three varieties of bacilli simultaneously, the second group first received inoculations of *B. typhosus*, and after an interval of two months, inoculations of *B. paratyphosus* A, and *B. paratyphosus* B simultaneously, the third group first received inoculations of *B. paratyphosus* A, and *B. paratyphosus* B simultaneously, and after an interval of two months inoculations of *B. typhosus*. These experiments showed that when a *mixed "triple vaccine"* of *B. typhosus* and the paratyphoid bacilli was used, the immunity produced (as measured by the agglutinins) obtained for each of its constituent bacilli was *at least as good as and very often greater* than, that obtained against any one of these organisms when it is employed alone in the same dosage. Secondly, when single vaccines are employed in succession and the immunizations are carried out independently, the response is greater to that vaccine which is introduced first. To the later immunizations with other microorganisms the specific response is almost always less intense. It has associated with it, however, as a secondary result, the production of a new rise of variable extent in the agglutinin-titer of the first immunization; i. e., if the individual is immunized first with *B. typhosus* and later with *B. paratyphosus* A and B, the immunity (as measured by the agglutinins) is greatest for *B. typhosus* and less for *B. paratyphosus* A and B, and vice versa.

The results of these experiments have answered the objection raised by some that the inoculation of paratyphoid A and B bacilli simultaneously with *B. typhosus*, in triple vaccines, might reduce the typhoid immunity. If anything,

when *B. paratyphosus* A and B are inoculated simultaneously with *B. typhosus*, in triple vaccines, the titer of the typhoid agglutinins is raised.

In these experiments it was found that in man a triple vaccine made so that 1.0 c.c. contained 1,000 million *B. typhosus*, 500 million *B. paratyphosus* A, and 500 million *B. paratyphosus* B, 0.5 c.c. of which was inoculated subcutaneously, followed after an interval of 10 to 16 days by the inoculation of 1.0 c.c., gave excellent results.

These methods of triple vaccination were adopted by the British Army in January, 1916, and have been made compulsory. A third inoculation of 1.0 c.c. of triple vaccine after an interval of 10 to 16 days would, in all probability, be even more efficacious but the exigencies of the training and mobilization of troops have made this impractical in many instances and so it has not been adopted.

The reaction to this dosage was in every instance not more severe than that to the usual vaccination against *B. typhosus* alone, namely the inoculation of 500 million *B. typhosus*, followed after an interval of 7 to 14 days by 1,000 million *B. typhosus*.

Reactions to typhoid and triple vaccines are usually noticeable only in those individuals who have previously suffered from typhoid or paratyphoid fevers, or those individuals who react severely to such infections as "common colds." The local reaction in such individuals consists in redness at the point of inoculation and slight stiffness of the arm. The general reactions are a feeling of sleepiness, slight headache and rise of temperature and occasionally some general malaise. In the vast majority of individuals no local or general reactions are produced, especially if rest and freedom from arduous duty are granted to the individual on the morning following the vaccination.

It has been noted that certain typhoid vaccines are more liable to produce more severe reactions than others. The reason for this is as yet unknown but it is usually found that fresh vaccines made in accordance with the technic described later (which is essentially that of United States Army vaccines) do not cause more than a moderate reaction. Kilgore<sup>12</sup> has shown, in regard to typhoid vaccines, that the use of army vaccine and sensitized vaccine sediment (Gay and Claypoole)<sup>13</sup> if the preparations are fresh makes little if any difference in the average local and temperature reactions.

Kilgore and Meyer<sup>14</sup> state that in comparison with army vaccine, sensitized vaccine sediment produces small amounts of agglutinins but an equal amount of complement-fixing antibodies.

Garbat and Meyer<sup>15</sup> found the sera of rabbits immunized with sensitized vaccines were highly protective (more so than those of animals treated with non-sensitized preparations) and yet these potent sera contained but small amounts of agglutinins and complement-fixing antibodies.

However, it has not as yet been decisively demonstrated in man that sensitized vaccines have any great advantage over the army vaccine that has been usually used in this country and abroad.

The length of time during which this triple vaccination will provide immunity has not, as yet, been definitely determined, for a longer period than one

year. In all probability, however, immunity is present a more prolonged time and a two year interval before reinoculation is usually adopted.

Official figures for the number of cases of typhoid and paratyphoid fevers in the British Army since the introduction of prophylactic vaccination with "triple vaccine" are not obtainable, but one high authority,<sup>16</sup> in December, 1916, one year after the adoption of triple vaccination, stated that there were practically no cases of typhoid and paratyphoid in the British Army. The withdrawal from the Gallipoli Peninsula is not accountable for this sudden decrease, for the Salonica and Balkan campaigns are in a territory nearly as unhealthy.

The method of making triple vaccine adopted in our experiments was briefly as follows: stock cultures of *B. typhosus*, *B. paratyphosus* A, and *B. paratyphosus* B were grown on agar, either in tubes or Roux bottles, for 24 hours at 37° C. The growth was then washed off with normal saline to which 0.5 per cent of phenol had been added. Each bacillary suspension was killed by heating in a water bath at 45° C. for 30 minutes and then gradually raising the temperature to 56° C. and maintaining it at that level for one hour. The suspensions were then proved sterile (both culturally and by inoculation into rabbits) and then counted (either by Wright's blood film method or by a Zeiss bacterial counting chamber). The suspensions were then diluted and mixed so that 1.0 c.c. contained 1,000 million *B. typhosus*, 500 million *B. paratyphosus* A and 500 million *B. paratyphosus* B. This triple vaccine was again proved sterile (culturally and by rabbit inoculation).

Prophylactic vaccination against *B. typhosus*, *B. paratyphosus* A, and *B. paratyphosus* B will eliminate practically all cases of infection with these three organisms; a few cases will still occur, the majority, as shown by experience, because some of the troops have failed to be vaccinated, through "conscientious objections" or through oversight; and a certain minimum in whom the vaccination does not produce sufficient immunity.

To differentiate these cases, which are practically always mild, from other pyrexias, an accurate quantitative method of serologic diagnosis must be used.\* The agglutination test when quantitatively performed by an accurate technic<sup>17</sup> can undoubtedly be made to give as valuable results in inoculated as in uninoculated persons: for although it is true that a single positive Widal reaction, even when done quantitatively, in the case of a person suspected of typhoid or paratyphoid fever who has previously been inoculated with triple vaccine, may be of no value, yet by recording the highest dilution in which marked agglutination occurs (that is, the titer of agglutination of the patient's serum) and repeating this quantitative determination once or twice at intervals of four days, one can readily arrive at a correct diagnosis by noting whether or not any appreciable alteration occurs in the titer of the patient's serum at the successive examinations (that is, noting whether the agglutinating power of the serum has risen or fallen during the period of observation). For one can state with confidence that if either a marked rise or a marked fall in the agglutination titer has taken place, the person is suffering from active typhoid or paratyphoid infection, since it has

\*For the first 8 days a blood culture is a reliable method of diagnosis, but after this period, a positive blood culture is rare, and positive stool and urine cultures are usually not obtained in more than 60 per cent of the cases.

been shown<sup>18</sup> that in active typhoid and paratyphoid infections the agglutination titer of the serum increases for about the first three weeks (Dreyer), after which it falls, at first rapidly, and then much more gradually toward its original level. The rate of fall progressively diminishes, so that after about two months, successive observations made at short intervals, will probably exhibit no appreciable change.

The agglutination technic by means of which this accurate analysis has been rendered possible is the quantitative macroscopic method introduced by Dreyer<sup>19</sup> in 1904, of which this brief description issued from the Department of Pathology, University of Oxford, is appended.

#### DIRECTIONS.

##### USE OF STANDARD AGGLUTINABLE CULTURES IN TESTING THE AGGLUTINATING POWER OF A SERUM.

I. *Technic*.—Take a stand\* containing 15 agglutination tubes in 3 rows of 5 each, and a dilution tube. (See note on size of agglutination tubes on p. 614.)

With the proper dropping pipette measure out into the dilution tube 54 drops of normal saline solution, 0.85 per cent sodium chloride, in distilled water (where the water supply is pure, tap-water can be used instead of saline solution) by means of gentle pressure on the teat.

Wash the pipette with distilled water.

Dry out the pipette with successive quantities of absolute alcohol, followed by successive quantities of ether, and get rid of the ether.

Take up the serum to be tested into the dried pipette. Measure out 6 drops of the serum into the dilution tube already containing the 54 drops of saline solution, thus obtaining a dilution of 1 in 10. Mix thoroughly.

Carefully wash out the pipette.

With the pipette measure out into each row of tubes as follows:

Number of tube.	Drops of Normal Saline Solution.	Drops of Serum Dilution 1 in 10.	
1	0	10	to each tube in row 1 add 15 drops of <i>B. typhosus</i> Standard Agglutinable Culture.
2	5	5	
3	8	2	to each tube in row 2 add 15 drops of <i>B. paratyphosus</i> A Standard Agglutinable Culture.
4	9	1	
5	10	0	to each tube in row 3 add 15 drops of <i>B. paratyphosus</i> B Standard Agglutinable Culture

At each stage of the procedure the pipette is carefully washed and dried out with successive quantities of absolute alcohol followed by successive quantities of ether.

Shake each tube thoroughly in order from right to left, i.e., beginning each row with the highest dilution.

Place the stand for 2 hours in a water-bath at 50°-55° C. (not in dry air).

In Tube 1 of each row the serum acts in a dilution of 1 in 25.

" 2 " " " 1 in 50.

" 3 " " " 1 in 125.

" 4 " " " 1 in 250.

Tube 5 containing no serum is control against spontaneous agglutination

If the limit of agglutination is not reached within this series, higher dilutions are followed out in a similar manner.

Thus, for example, 57 drops of normal saline solution plus 3 drops of a 1 in 10

\*Stands, dropping pipettes, agglutination tubes, etc., can be obtained from Messrs. Baird and Tatlock, Hatton Garden, E. C. London. If the larger agglutination tubes are used the quantities of saline, serum and culture are measured in tenths of a c.c. in the same proportions as the drops.

serum dilution will give a serum dilution of 1 in 200, and, using the same quantities as before, one has the serum acting in dilutions of 1 in 500, 1 in 1000, 1 in 2500, and 1 in 5000. And similarly for higher dilutions.

The tubes are examined after 2 hours at 50°-55° C. followed by 15 minutes standing at room temperature. The reading is taken by comparing each tube in succession with the control tube, and is preferably made by means of artificial light against a black background. If daylight is used, the tubes inspected should be partly shadowed by passing a finger up and down behind them.

The highest dilution in which marked agglutination (without sedimentation) can be detected by the naked eye is *standard agglutination*. But owing to the rate at which the dilution increases in the series of tubes employed it will commonly happen that no tube in the series exhibits *standard agglutination*. If this be so it will be found in looking along the series that while one tube shows strong agglutination with sedimentation the next succeeding tube shows no agglutination at all or only a trace. In such cases *standard agglutination* lies approximately midway between the two dilutions.

Should a more precise determination of the limits of agglutination be required, it can be obtained by using a stand of 12 tubes with the series of quantities given in the table contained in the directions for Preparation and Standardization of Agglutinable Cultures.

(If the stand is left at the room temperature, 16 to 24 hours must be allowed before the reading is taken, but the reaction is not then so sharply defined. In this case the highest dilution in which a definite flocculent sedimentation appears corresponds approximately to *standard agglutination*.) The same is true for 8 hours in an incubator at 37° C.

When the standard degree of agglutination ("*standard agglutination*") occurs with Standard Agglutinable Culture in a serum dilution of 1 in  $x$ , then  $x$  divided by the figure given on the label of the Standard Agglutinable Culture employed gives the number of "*standard agglutinin units*"\* contained in 1 c.c. of the serum examined.

Thus if *standard agglutination* occurs in a dilution of 1 in 1000 and the number on the label is 2.5, then  $\frac{1000}{2.5}$ , i.e., 400, is the number of Standard Agglutinin Units contained in 1 c.c. of the serum examined.

For uniformity and simplicity in recording results they should be expressed in *standard agglutinin units*.

2. *Diagnosis*.—A. In non-inoculated persons who have not had typhoid (or paratyphoid) fever, agglutination in a dilution of 1 in 25 justifies a strong suspicion of typhoid (or paratyphoid) infection. But the test must be applied again in the course of a few days to ascertain whether there is any change in the titer of agglutination. Marked agglutination in a dilution of 1 in 50 or more is (nearly always) diagnostic of active typhoid (or paratyphoid) infection.

A *non-inoculated* "carrier" will normally show no important change in the titer of his serum on repeated examination at short intervals.

B. Inoculated persons if quite recently inoculated will usually show a high titer of specific agglutination. A rapid rise in titer sets in within two to four days of inoculation. This is followed by a fall at first rapid, but subsequently becoming very slow, so that a relatively high titer is maintained for a long period (even for years). During this period examinations made at intervals of a few days give practically identical readings.

It follows that in the case of inoculated persons the diagnosis of active typhoid (or paratyphoid) infection will require two or more successive examinations of the serum.

(a) If the individual is suffering from active *typhoid* infection his titer of typhoid agglutination will exhibit the usual rise and subsequent regular fall seen in non-inoculated subjects, but starting from and returning towards the higher base line of inoculated persons.

(b) If the individual is suffering from active *paratyphoid* infection one of three things may occur as regards his *typhoid* agglutination titer namely:

1. No appreciable change may occur in the titer of typhoid agglutination.
2. A relatively slight rise may occur, followed by a fall towards the former level.
3. A marked rise may occur synchronous with the rise in paratyphoid agglutination titer, and subsequently followed by the usual fall towards the former level.

Meanwhile the titer of *paratyphoid* agglutination runs the normal course of rapid



rise to a maximum (usually exceeding the maximum typhoid titer) followed by a fall, at first rapid and then slower as already described for typhoid subjects, and falling *below* the persistent base line of typhoid agglutination of inoculated persons.

C. In the case of mixed infections whether in inoculated or noninoculated persons the agglutinin curves for the different infecting organisms are usually not synchronous, and they pursue their ordinary course independently of each other.

\**Note.*—The standard agglutinin unit is that amount of agglutinating serum which when made up to 1 c.c. volume with normal saline solution causes standard agglutination on being mixed with 1.5 c.c. of a particular standard agglutinable culture and maintained at 55° C. for 2 hours in a water-bath followed by 15 minutes at the room temperature.

*From the Department of Pathology, University of Oxford,  
on behalf of the Medical Research Committee.*

In Great Britain and Denmark this technic has now very largely superseded the older microscopic method. Its application and the use of the standardized agglutinable cultures,<sup>20</sup> which have been issued since July, 1916, on behalf of the Medical Research Committee (England) to British military and naval hospitals, have rendered it a simple matter to determine accurately the degree to which the serum of any given individual will agglutinate the *Bacillus typhosus* or the paratyphoid bacilli.\* A description of the mode of preparation and standardization of these cultures is sent out from the Oxford laboratory in the following form:

*Preparation.*—The bacillus (*B. typhosus*, *B. paratyphosus*, etc.) is grown for twenty-four hours at 37° C. in ordinary veal peptone bouillon in large Erlenmeyer flasks partly filled (1 liter of bouillon in a 1½ liter flask).

The bouillon is titrated against phenolphthalein, and two-thirds of that amount of sodium hydrate which would render it neutral to phenolphthalein is added before the final boiling and filtration.

Before use the flasks of bouillon are sterilized in the autoclave at 115° C. for *not more* than fifteen minutes, and are then tested for sterility by incubation at 37° C. for forty-eight hours.

They are inoculated with a few drops each from a twenty to twenty-four hour old bouillon culture of the bacillus (*B. typhosus* or *B. paratyphosus*, etc.).

The culture used should be one which has been subcultivated daily in bouillon for one or two weeks (or longer). This continued subcultivation has the effect of increasing its agglutinability and diminishing any tendency to spontaneous agglutination.

At the end of twenty to twenty-four hours' growth at 37° C., the flasks are well shaken, and to each is added 0.1 per cent (1 c.c. per liter) of commercial (40 per cent) formalin. They are again shaken and placed in a cold chamber in the dark at about 2° C.

At intervals on the same day and on subsequent days for four or five days the flasks are again thoroughly shaken and *replaced at once in the cold chamber*.

After three or four days they will be found to be absolutely sterilized. Should it happen that the bacterial suspension is not entirely homogeneous it may be shaken for some hours in a mechanical shaker, or may finally be filtered through sterile cotton-wool.

*Standardization.*—The process of standardization consists (A) in making up the killed culture to an opacity as nearly as possible identical with that of the Standard Agglutinable Culture, (B) in measuring its agglutinability as compared with the Standard Agglutinable Culture by the use of Standard Serum.

A. The killed culture is diluted to the required degree with normal saline solution to which has been added 0.1 per cent of commercial (40 per cent) formalin.

A. *Details of Method.*<sup>22</sup>—*Apparatus.*—Almost any variety of dwarf test tube or agglutination tube may be used. It is essential to have at hand a fairly large supply of tubes, so that a selection may be made of tubes of practically equal internal diameter. The tubes must be quite clean and their surface must be free from scratches. When the

\*Any laboratory can be readily equipped to make and issue in large quantities these standard agglutinable cultures of typhoid and paratyphoid bacilli. They may also be obtained by writing to the Standards Department, Department of Pathology, Museum, Oxford, England.

greatest possible accuracy is desired, it is best to use the larger\* form of tube and to measure the fluids with a 2.0 c.c. pipette, accurately graduated in hundredths of a cubic centimeter. But for ordinary purposes the small\*\* agglutination tubes may quite well be employed, and the dilutions made with a dropping pipette. In this latter method there is a certain error due to the difference in size of the drops of 0.85 per cent NaCl solution and of culture. About 17 to 18 drops of culture are equal to 15 drops of saline. But in dealing with small volumes of fluid, it is open to doubt whether measurements with a small graduated pipette are any more accurate than this.

*Technic.*—(For the smaller tubes the quantities of saline, serum and culture are measured in drops; for the larger tubes in tenths of a c.c.) Two sets of eleven gauged tubes are placed in a suitable stand. Into the tubes of each set the different fluids are measured as follows:

Tube	Saline	Culture
1	0	20
2	4	16
3	8	12
4	10	10
5	12	8
		Culture diluted 1 in 2
6	6	14
7	8	12
8	10	10
9	12	8
10	14	6
11	16	4

This series of dilutions is similar to that described for the serum dilution in the standardization of agglutinable cultures, only multiplied by two. It will be seen that the range of dilutions in the series is from 1/1 to 1/10, and that the progressively diminishing quantities of culture are always made up to the same volume with saline. The mixtures in the tubes are well shaken with the finger over the mouth of the tube, and the readings are then taken.

*Readings.*—Artificial light and a black background are essential. It has been our custom to use a frosted electric bulb with a large green shade, and some pieces of dull black boarding or paper placed on the table below the lamp and propped up behind it. A darkened room, though not absolutely necessary, adds considerably to the ease and accuracy of the readings. The lamp shade is inclined so that the bulb is just hidden from the eye, and the tubes to be compared are held up against the edge of the shade. Before reading, the outside of the tubes must be wiped with the moistened corner of a glass-cloth and then polished with the dry part of the cloth. From one series a tube is chosen at random which we call the "standard" tube for the first reading. With this the tubes of the other series are compared one after the other until one is found which matches it in turbidity. It will frequently be found that there is no tube in the second series which gives an exact match, but that there is one tube which is a little more turbid while the next lower is a little less. In this case the standard tube is recorded as lying between those two tubes of the other series.

Moreover it is usually possible to judge whether it is nearer to one than to the other. If so, we make a mark or marks under the number of the tube to which it is nearer. Five or six readings should be made in this manner, taking a number of tubes in succession as standard. It is well to read in both directions; i.e., to take tubes from each series as standard. Now, since we know the dilution of the culture in every tube, it needs only a simple calculation to arrive at the relative turbidity of the two cultures. Records of typical routine estimations are given in Table I. Moreover, twelve tubes may be used with advantage instead of eleven.

Thus: Culture (c.c.) 1.0, 0.8, 0.6, 0.5, 0.4, 0.35, 0.3, 0.25, 0.2, 0.17, 0.13, 0.10. Water (c.c.) 0.0, 0.2, 0.4, 0.5, 0.6, 0.65, 0.7, 0.75, 0.8, 0.83, 0.87, 0.9. The range of dilution is the same as before; and the only difference is that the series is fuller by one tube at the right-hand end, which renders readings in this neighborhood more accurate.

\*A thin-walled dwarf test tube about 7.0 cm. long and 0.95 to 1.0 cm. in diameter.

\*\*A smaller "agglutination tube" 5.7 to 5.8 cm. in length, having an internal diameter of 5.5 to 6.2 mm.

Moderate variations in the color-intensity of the fluids do not appreciably affect the accuracy of the readings, for the progressive dilution renders such differences negligible at an early point in the series. If one of the fluids under comparison is so thick that the range of dilutions described proves insufficient to allow a comparison with the other fluid; then a further series of tubes must be set up in precisely the same manner, but using the thicker fluid diluted (1 in 10, or 1 in 20) instead of at full strength.

In practice it is often convenient to make a rough estimate of the relative thickness of the fluids before proceeding to the complete test. This is easily done by taking a small quantity of the two fluids in two tubes of equal caliber, and diluting the thicker fluid until the two show approximately equal turbidity. From this rough test it is easy to make an approximate calculation of the relative thickness of the fluids. If one fluid appears to be four or five times thicker than the other, it is best to dilute it suitably before putting up the test series.

TABLE I.

ESTIMATION OF RELATIVE TURBIDITIES OF FLUIDS A AND B.

Readings		Actual quantities of culture in the matched tubes		Ratio— quantity of A quantity of B	Deviation from mean in per cent
Series A Tube	Series B Tube	A	B		
5 matches	9	0.40 c.c.	0.20 c.c.	2.00	0.50
2	5	0.80 c.c.	0.40 c.c.	2.00	0.50
6	10	0.35 c.c.	0.17 c.c.	2.06	2.49
3	7	0.60 c.c.	0.30 c.c.	2.00	0.50
7	10-11	0.30 c.c.	0.15 c.c.	2.00	0.50
				Mean 2.01.	Mean error, 0.90%.

Calculation for diluting B to the same turbidity as A:

1.0 c.c. of B = 2.01 c.c. of A.

Therefore, to each 1.0 c.c. of B add 1.01 c.c. of diluting fluid.

B. To measure the agglutinability of the killed culture thus diluted, proceed as follows.

Take two stands and place twelve agglutination tubes in each. Prepare (1) a dilution of standard agglutinating serum of such strength that each cubic centimeter contains from 4 to 8 standard agglutinin units, and from this prepare (2) a second dilution of half that strength.

With the pipette measure out

Drops of normal  
saline solution.

Serum dilution.

Into Tube 1 of each stand 0						10 drops* of dilution 1			
"	"	2	"	"	2	8	"	"	"
"	"	3	"	"	4	6	"	"	"
"	"	4	"	"	5	5	"	"	"
"	"	5	"	"	6	4	"	"	"
"	"	6	"	"	3	7	"	"	2
"	"	7	"	"	4	6	"	"	"
"	"	8	"	"	5	5	"	"	"
"	"	9	"	"	6	4	"	"	"
"	"	10	"	"	7	3	"	"	"
"	"	11	"	"	8	2	"	"	"
"	"	12	"	"	10	0	"	"	"

(To each tube of one stand is added 15 drops of *Standard Agglutinable culture*, and to each tube of the other stand 15 drops of the *killed culture* under standardization.)

\*If the larger tubes are used these quantities are measured in tenths of a c.c. in the same proportion as the drops.

At each stage of the procedure the pipette is carefully washed and dried out with successive quantities of absolute alcohol followed by successive quantities of ether.

The stands are placed for two hours in a water bath at  $50^{\circ}$  to  $55^{\circ}$  C., then allowed to stand for fifteen minutes at room temperature and a reading subsequently taken by selecting in the series made with standard agglutinable culture the tube which exhibits standard agglutination (the highest dilution in which marked agglutination, without sedimentation, can be detected by the naked eye), and ascertaining which tube in the other series shows the same degree of agglutination. Should the tube be the same in each series, the agglutinability of the killed culture is clearly equal to that of the standard. If not the same, the degree of agglutinability of the killed culture is now readily determined.

Thus suppose that Tube 5 in the Standard series corresponds to Tube 2 in the other series. The Standard Agglutinable Culture is twice as agglutinable as the killed culture under standardization, since only half the quantity of serum has been required to agglutinate it to the same degree.

Hence, if any given serum presented for examination is found to agglutinate this particular killed culture in a dilution of, say, 1 to 500, then 500 multiplied by 2 and divided by the figure given on the label of the Standard Agglutinable Culture is the number of Standard Agglutinin Units in 1 c.c. of the serum examined.

Or, again, if the killed culture were, say, 1.3 times as agglutinable as the Standard Agglutinable Culture, then in the same example as above, 500 divided by 1.3 and again divided by the figure given on the label of the Standard Agglutinable Culture is the number of Standard Agglutinin Units in 1 c.c. of the serum examined.

As a matter of routine, quantitative agglutination tests are made simultaneously against *B. typhosus*, *B. paratyphosus* A, and *B. paratyphosus* B, and the highest dilutions in which marked agglutination without sedimentation occurs are recorded. Since, wherever triple inoculation has been made a routine practice, it is found that about 99 per cent of the inoculated individuals give a positive agglutination reaction (Widal) against *B. typhosus*, *B. paratyphosus* A, and *B. paratyphosus* B for at least a year, therefore a second specimen of blood must be taken four or five days later and the test repeated. If the highest dilution in which marked typhoid or paratyphoid A or B agglutination occurs on the second occasion is approximately the same as that found on the first occasion, the case is not one of typhoid or paratyphoid A or B fever, and the agglutination is due to the triple inoculation alone. For it has been shown that after about the third month of antityphoid and paratyphoid A and B inoculation, the titer of the agglutination due to inoculation is practically stationary if examined at intervals of only a few days, and that tests made at intervals of even weeks will show only a small gradual decrease in titer.<sup>21</sup> If, on the other hand, the highest dilution of marked agglutination for any of the bacilli (typhoid and paratyphoid A and B) in the second test is either markedly higher or markedly lower than at the first examination, one can state that the case is one of active infection with the bacillus whose titer showed the alteration, i. e., either *B. typhosus* or *B. paratyphosus* A, or *B. paratyphosus* B.

#### CONCLUSIONS.

1. Prophylaxis against typhoid and paratyphoid A and B is absolutely necessary for armies operating in the United States and Europe.

2. Mixed "triple" vaccine containing in 1.0 c.c.; 1000 million *B. typhosus*, 500 million *B. paratyphosus* A and 500 million *B. paratyphosus* B; 0.5 c.c. of which is injected subcutaneously, followed at an interval of 7 to 16 days by the injection of 1.0 c.c. at a further interval of 7 to 16 days is advisable.

3. Accurate quantitative agglutination tests with standard agglutinable cultures made at four day intervals is the best method of diagnosis of typhoid and paratyphoid infection in inoculated individuals.

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## THE SIGNIFICANCE OF LAMBLIA INTESTINALIS IN STOOL EXAMINATIONS\*

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THAT certain protozoal parasites have a definite pathogenicity in some forms of intestinal disturbances is acknowledged by practically all clinicians. The role played by the flagellate, *Lambliia intestinalis*, is still an open question if one is to judge by the diversity of opinion expressed in the literature on the subject. In the writings of Braum, Park and Williams, Barker, Besson, Allbutt and Rolleston, and many others, it is tersely stated that they are nonpathogenic. Other writers, among them Emerson, Stiles, Rodenwaldt, MacNeal, and Neveu-Lemaire, consider that their part as an etiologic factor in chronic diarrhea is not entirely settled, but that they may have much to do in prolonging symptoms when the disease is once established. On the other hand, Daniels, Brumpt, and Stitt each ascribe a pathogenic role to these organisms, the last mentioned author stating that they are "responsible for a chronic and intractable diarrhea, an infection only minor in importance to amebic infection."

We have not attempted to review the literature fully, but may state that in recent articles on the subject *Lambliia intestinalis* has been considered pathogenic by the following authors: Du Bois and Toro, Wenyon, Russell, Kennedy and Rosewarne, Porter, Fantham, Fantham and Porter, and Mathis.

In tropical regions this parasite is encountered very frequently in stool examinations. In our country, especially in northern latitudes, it is not found commonly. The cysts are easily recognized, and when present are found in great numbers if the patient has had the usual preparation for examination for endamebas, i.e., one-half to one ounce of salts before breakfast. It is quite usual, when cysts are present, to find free, motile forms the next morning after the patient has taken a second dose of salts.

In stool examinations made for more than 6,000 patients during the past six years, we have found this parasite in only a trifle more than 1 per cent of the cases. The patients came from all parts of the United States, but chiefly from the northern states; three were from Canada.

This report, then, is based on 66 cases in which *Lambliia intestinalis* has been found in stool examinations. In 41 persons this was the only organism that might account for the complaint. In the remaining 25 cases there were other organisms, or pathologic conditions which presumably, in some instances at least, accounted for the complaint.

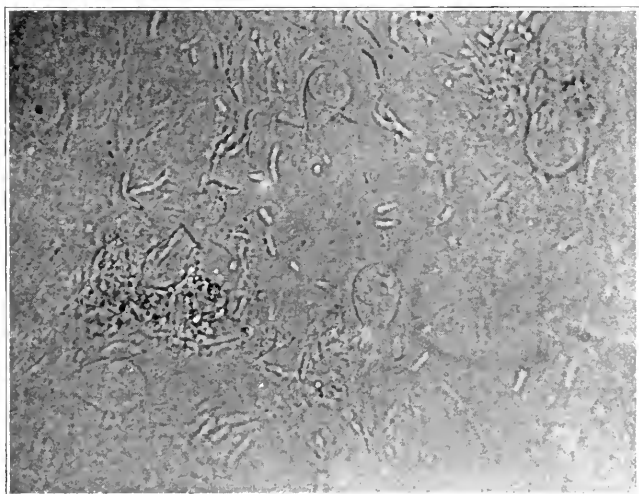
In studying the histories of these cases we find there is apparently no syndrome which is diagnostic without a stool examination. A history of preceding, or present diarrhea, usually without blood or mucus, often with most or all of the stools in the early morning hours, with rumbling and rolling in the intestines, indefinite pain slightly more to the right side, an "all in" feeling, and nervous indigestion are all suggestive symptoms, but in no way diagnostic. After

\*From the Mayo Clinic, Rochester, Minn.

the initial attack of diarrhea, constant looseness, alternate constipation and diarrhea, or constipation may remain.

As lamblias have been held to be nonpathogenic by most observers, no attention has been paid to their presence in the stools, and little or no treatment has been directed toward their removal. Possibly for this reason many of our patients give long histories. Nineteen of the series of 41 patients gave a history extending over five years, 11 of them having a constant looseness or severe diarrhea. Eighteen of the series had constant looseness varying from 3 to 20 stools daily; 8 others had spells of diarrhea; 3 had normal stools; and the remainder were constipated.

As the lamblias inhabit the upper intestinal tract, we would not expect free blood in the stools or much inflammation in the colon. Six of the 41 patients had seen some free blood (one of these had bleeding piles), 18 expressly stated



Photomicrograph of *Lamblia intestinalis*.

that no blood had been passed, and in the other cases no mention of blood was recorded. In the 25 cases in which there were complications, 5 patients had noted blood and 13 had not.

Proctoscopic examinations were made for 10 of the 41 patients. Six had normal bowels, 3 a slight proctitis and 1 a diffuse colitis without ulceration. Of 5 patients in the series of 25 who had proctoscopic examinations, 2 had normal bowels, 1 a mild colitis, and 2 ulceration due to amebas.

Twelve of the series with lamblias alone had examinations of the stomach. In 9 the acids were normal, and in 3 there was absence of hydrochloric acid. Six patients had barium roentgenographic examinations of the stomach, and in all the findings were negative. Blood examinations were made in the majority of the cases, but showed no change in the eosinophiles and in only two patients of the larger series was the hemoglobin reduced (45 per cent).

Since the symptoms definitely indicated some trouble and in many cases were of long duration, and since lamblias were not considered pathogenic, other

organs than the intestines were suspected to be the site of the trouble. Ten of the 41 patients were operated on, and 3 of the other group of 25. One of the 10 had carcinoma of the rectum and was operated on five years after we first found the lamblias. One was operated on for enlarged spleen which later turned out to be a lymphosarcoma. Five had appendicectomies; 4 were not helped; the fifth had had a history of several definite attacks of appendicitis, and was relieved of that trouble. On 2 cholecystectomies were done without relief; and in 1 the gall bladder was the surgical objective, but was found normal. In this case a pericolitis was considered the cause of the trouble. Three other patients had surgical diagnoses—1 a diagnosis of appendicitis, 1 of cholecystitis, and 1 of duodenal ulcer—but were not operated on.

Of the three operations in the second group, 1 appendicectomy gave no relief, 1 cholecystectomy and appendicectomy gave no relief, and 1 appendicectomy on a patient with a history of ten definite attacks of appendicitis gave relief.

Nine, only, of the 41 patients were treated in the clinic, and these for a short time only. Thymol and methylene blue were used. Thymol, given in 6 instances, relieved the symptoms temporarily, and removed lamblias from the stools in 4 cases. However, only one stool test was made after treatment in each instance. In 1 case a severe diarrhea was stopped and the patient gained 27 pounds, but four months later the symptoms recurred. In another case the diarrhea stopped and the hemoglobin rose from 45 per cent to 68 per cent in one month. Methylene blue was of benefit in only 1 case.

In 10 of the cases in which amebas were a complication, treatment consisted of emetin, ipecac and coal oil enemas, the lamblias being disregarded. Five of these were symptomatically relieved, though in 1 case there was a recurrence in six weeks. In none did this treatment remove the lamblias. In 1 instance in which both lamblias and amebas were present, the patient was treated with thymol and coal oil enemas; both organisms were removed, and the patient was relieved of the diarrhea.

Thus, it is likely that when the pathogenicity of these parasites has been disregarded, the diagnosis and treatment of many patients has not been correct; and that when *Lamblia intestinalis* has been considered the cause of the condition, and treatment has been instituted against it, better results have been obtained.

#### CONCLUSIONS.

1. *Lamblia intestinalis* should probably be considered a pathogenic parasite.
2. We have obtained no definite syndrome in our series.
3. The removal of lamblias is difficult.
4. The best results have been obtained from thymol medication.

#### TWENTY-FIVE CASES OF *LAMBLIA INTESTINALIS* WITH OTHER COMPLICATIONS.

<i>Complications.</i>	<i>Cases.</i>
<i>Endameba histolytica</i>	9
<i>Endameba histolytica</i> and active pulmonary tuberculosis	1
<i>Endameba histolytica</i> and history of passing recent tapeworm and hookworm	1
Actively motile ameba (not diagnosed)	1
Nonmotile ameba	2



Encysted <i>Endameba coli</i>	2
<i>Endameba coli</i>	2
Ova of tenia	1
Active pulmonary tuberculosis	4
(1, with tuberculous peritonitis)	
(1, tubercle bacilli found in stools)	
(1, pulmonary symptoms came eighteen months after abdominal symptoms)	
Pernicious anemia	2

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## CHLOROMA: WITH REPORT OF A CASE\*

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IN recent years, there has been extensive literature presented upon the group of diseases associated with the blood-forming organs. This has, undoubtedly, been stimulated by the confusion of classification and insufficient knowledge of their etiological factors. Considerable evidence has been accumulated to show that many of the specially named blood diseases are closely related to each other, even to such an extent that some observers believe that they are transmutable. Cases closely simulating the one recently reported by Warfield and Kristjansen which showed the transition of lymphosarcoma to acute lymphatic leukemia and thence to Hodgkin's disease lend much to this latter view.

Chloroma is very closely related to the acute leukemias and probably should be considered under them. The term chloroma was first used by King in 1853, when he applied it to a case of greenish tumors of the head, coming under his observation four years previously. The first report of this condition is attributed to Allan Burns, who described it in 1823. He, however, did not attempt to name the condition. His case was one of greenish yellow tumor masses occurring about the bones of the head. Dock reported the seventeenth case to which reference is found in the literature in 1893 and briefly reviewed the sixteen previous cases. Since then, sixty-nine cases, classified as chloromata, have been reported.

From the cases which have been described it would seem that the term is not entirely appropriate if the condition is to be regarded as a separate pathological entity, inasmuch as cases have occurred in which all of the clinical and pathological features ascribed to chloroma have been present except for the green color, and others where only some of the nodules, glands or blood clots showed the green color at autopsy. "Nonchloromatous leukemia" has at times been applied to the former type of cases, but it also would appear to be a misnomer. Sternberg, in his classification of the diseases of the hematopoietic organs, describes under hyperplasia of lymphoid tissues with leukemic blood and having tumors originating in various situations and invading tissues, leukosarcoma. If the tumor masses of such a condition are of green color, the name chloroleukosarcoma is applied. Likewise such a condition with leukemia of myelogenous origin is named chloromyelosarcoma. These terms seem appropriate if they are used to describe a form of acute leukemia with an origin in the bone marrow, in which the cells are so malignant in type as to erode the bones to form tumor masses upon their surfaces, some or all of these masses presenting a greenish color. In most of the cases described as chloroma previous to that of Klein and Steinhaus, reported in 1904, the cell type was thought to be of lymphocytic origin and the condition was classified by various writers as a form of lymphatic leukemia, lymphosarcoma or a transitional stage between them. Since then, however, the majority of authors have classified the cell as of myelogenous origin and the condition as a form of acute myelogenous

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leukemia. Both Treadgold and Burgess convey the idea that it is probably always of myelogenous origin. The increase in the proportion of reported cases of myelogenous origin is probably due, as suggested by Burgess, to the more thorough examination of the cells both morphologically and biologically, as by the oxydase reaction, which he thinks has shown that certain of the cases formerly classed as acute lymphatic leukemia must be considered as myelogenous. Forman and Warren recently reported a case of myeloma, many of the cells of which, by ordinary staining methods, seemed to be plasma cells. The cells, however, contained oxydase granules, showing them to be of the myelogenous series. Without such examination this case probably would have been called plasmoma.

That the condition is always due to a primary involvement of the bone marrow is shown by the fact that in practically all cases in which the bone marrow was examined it was found altered; that the location of the tumor masses attached to the bones is always found in the short bones and those of the skull, in the marrow of which the hematopoietic function is still active; that the cells evidently have grown directly through the bones to form the masses, as the surfaces of the bones are eroded and the masses fairly well attached; and that the cells in a number of cases have been definitely proved to be myeloblasts and myelocytes since their protoplasm either showed granules by differential staining methods or oxydase granules upon application of the oxydase reaction.

The blood picture of an acute leukemia, concomitant with chloroma shows the same type of cell which is predominant in the green tumor, as well as in sections of the bone marrow. Burgess states that the highest leucocyte count was 1,880,000 per cubic mm. In a few cases no increase of white blood cells was noted and for these forms the term "aleukemic chloroma" or "chloromatous pseudoleukemia" was suggested. However, there is not sufficient evidence to introduce this terminology as indicating a distinct type of disease.

Although the terminology is confusing, it would seem that when the term chloroma is used, a type of leukemia is referred to in which there is the formation of green masses of immature white blood cells. The leukemia is probably always myelogenous in character and is due to a primary hyperplasia of the red bone marrow. The marrow cells develop to such a degree that they erode and grow through the bone to form green tumor masses upon its surface, as well as evidence metastatic power to enter the blood stream and lodge in distant organs, where they may multiply. Some or all of the cell collections, whether seen as tumors upon the surfaces of certain bones, as nodules in the organs, or occurring in the blood clots, bone marrow or glands, may show the greenish color at autopsy. Treadgold says, "Since it is usual in chloroma for a varying proportion of the lesions to show no green color, it is theoretically possible to get a fairly acute type of myeloblastic leukemia, bearing a general resemblance to chloroma, but without the green lesions."

The clinical and pathological features of most of the cases are similar. It occurs more frequently in children, particularly males. When not occurring in children, the most frequent age incidence is after the fourth decade. The patient generally complains of weakness, anemia, pain due to pressure of tumorous masses, and a tendency to hemorrhage. Exophthalmos is frequent, due to

green tumor formation in the orbital fossæ. The disease runs an acute course from weeks to months and so far as known is invariably fatal. Leukemia is present. At necropsy, the chief findings are flattened greenish tumors over the inner surface of the bones of the head, vertebræ, sternum, or ribs. The bony surface beneath is eroded and to it the tumor is attached. The marrow spaces are increased in size and the bony trabeculæ rarefied. Tumor nodules may be found in the various organs and some of the blood clots may have the greenish color. An absolute diagnosis cannot be made, except by encountering the green color in the tissues. This has been done in several cases during life by finding the presence of green nodules subcutaneously. Attempts to diagnose by the symptoms and location of masses is only conjecture as the same may occur in acute leukemia, lymphosarcoma and myeloma. The diagnosis was made in a case reported by Butler upon the following points; age 11 years, acute leukemia, exophthalmos and right-sided deafness. The exophthalmos in this case, contrary to expectation, was found not due to the size of the orbital tumor mass but to vascular engorgement. This shows how easy it is for one to err in the diagnosis of certain manifestations.

While the report of the two cases by Gould and Le. Wald is noteworthy, yet in the absence of autopsies and definite leukemia, their classification as chloroma is uncertain. Their diagnosis before death was made upon age (children), exophthalmos, periosteal and medullary changes of the bones, glandular enlargement, and microscopical picture of glands removed from the neck. No leukemia was present in the first case and the white blood count in the second case was only 16,500. Such a count could be present in lymphosarcoma or many infections.

The following case was under the service of Dr. J. A. Lichty to whom I am indebted for the clinical notes:

The patient, an adult male, age 36 years, was admitted to the Mercy Hospital, November 16, 1915. He complained of weakness and pain in the right chest and beneath the sternum. His family history was negative. There was previous history of measles, mumps, whooping cough, diphtheria, typhoid fever, malaria, lues and Neisser infection. His luetic infection had occurred seven years previously and for one year he had received mercury by mouth and injections. Cardiorespiratory and digestive systems were negative. On admission, physical examination showed a well developed male, not acutely ill. A moderate amount of anemia was present. The pupils were equal, regular in outline, symmetrical and reacted to light and accommodation. No exophthalmos or other ocular signs. Some purulent exudate was present over the left tonsil. Very little glandular enlargement present. The thorax showed a distinct depression over the right apex and subclavicular region and very little rotation of ribs of the right side on respiration. The percussion note over the right apex, right subclavicular region, and region lateral and inferior to the right scapula was impaired. No ascites. The liver was palpable at the costal margin. The spleen seemed enlarged to percussion but not palpable. Wassermann reaction for lues was negative.

#### HEMATOLOGICAL EXAMINATION.

	Nov. 17, 1915.	Nov. 20, 1915.	Nov. 22, 1915.	Nov. 26, 1915.	Nov. 30, 1915.	Dec. 7, 1915.
Hemoglobin	30			20	15	
Red blood cells	1,632,000			872,000	600,000	675,000
White blood cells	53,000	44,000	43,000	45,200	138,200	320,000
Myeloblasts	73	76		82	77	
Polymorphonuclears	9	11		11	7	
Transitionals	1				2	
Large lymphocytes	11	6		2	3	
Small lymphocytes	0	7		5	11	

*Blood Smears.*—The blood smears were stained by Wright's method. The white blood cells were greatly increased in relative proportion to the red blood cells. A number of nucleated reds were observed. The leucocytes varied in size from 8 to 21  $\mu$  in diameter. All of these cells, even the forms appearing as small lymphocytes, were pale staining. Sometimes the nuclei of the small lymphocytes showed vacuolation, often giving a rosette arrangement. The large mononuclear forms classified as myeloblasts were round or oval and had very pale, large, single nuclei, which occupied the greater portion of the cell so that only a relatively small rim of cytoplasm appeared at the periphery. The nuclei did not appear reticulated. The cytoplasm was faintly basophilic and slightly darker in staining quality than the nuclei. At the time when these smears were made there was no mention of specific granules in these cells. It is probable, however, that they were somewhat different from true myeloblasts, as a new examination of the smears one year later showed the presence of a small percentage of both eosinophilic and basophilic myelocytes. The granules of the polymorphonuclear leucocytes were very faint and could not be made out with the stain (Wright's) used. Possibly with better staining methods for granules these could have been demonstrated in some of the cells termed myeloblasts. Polymorphonuclear leucocytes were few in number. During life no examination was made for oxydase granules in the cells of fresh blood films. This was attempted some time after death, but the films were too old to demonstrate the reaction.

The clinical diagnosis was "myeloblastic leukemia." The patient died three weeks after admission to the hospital. During this time the white blood count as shown above increased gradually from 53,000 to 320,000 cells per cubic mm. An autopsy was performed 4 hours after death by Drs. W. W. G. MacLachlan and J. W. Fredette.

#### AUTOPSY.

The body was that of an adult male measuring 160 cm. in length. The body was fairly well developed, but poorly nourished. The skin surfaces were very pale. There was no rigor mortis and only slight lividity on the dependent parts. The pupils were equal and moderate in size. Scattered over both arms, upper left thorax and abdomen, were a number of tiny petechial hemorrhages. The thorax was well formed. The abdomen was slightly flattened. There were no scars on the penis. The anus was normal. There was slight edema of the ankles.

*Thorax.*—On both sides there were some fibrous adhesions over the posterior surface of the lungs and also at the apices. About 100 c.c. of clear straw-colored fluid was present in each pleural sac. The pericardial sac contained a clear straw-colored fluid in a quantity of about 100 c.c. In the fat over the pericardium were a number of petechial hemorrhages. An occasional hemorrhage was also noted on the parietal and visceral pericardium. Lying opposite the left apex of the lung beneath the parietal pleura and over the inner surface of the ribs and also on the bodies of the thoracic vertebrae there was a thick, quite green, firm, smooth tissue, forming a layer, which, although well attached to the bony structures underneath, could be peeled away leaving evidences of an eroded underlying bone. This tissue when removed measured 5x4x1.3 cm. Its pleural surface adjacent to the lung showed carbon pigmentation to a very marked degree, otherwise it was green. On section, the cut surface had a glassy, smooth appearance. There were some fibrous tags running between this tissue and the apex of the lung. The green color faded gradually after the removal of the tissue. The pleura covering the ribs showed many tiny petechial hemorrhages.

*Left Lung.*—Weight 630 gm. The posterior surface and the apex of the upper lobe showed a few dense fibrous adhesions. The lung was quite black in color showing much anthracosis. It felt moderately firm but crepitated in most places. Section through the lower lobe showed a cut surface, fairly dry, of grayish pink color, and with very prominent nodules of anthracotic pigment. The upper lobe was a little lighter in color and somewhat moister. At the apex, opposite the fibrous adhesions, was a small area of fibrosis in the lung substance. There was no calcification nor caseation noted here. The bronchi were free. The peribronchial glands showed marked anthracosis, but no evidence of tuberculosis.

*Right Lung.*—Weight 800 gm. There were some fibrous adhesions over the posterior portion of the lower lobe and on the diaphragmatic surface. The lung felt firm but crepitated in most places. The lower lobe, on section, showed a somewhat moist pinkish gray surface, glassy throughout, with numerous nodular areas of anthracosis. Petechial hemorrhages were also seen in the lung substance. There was no consolidation. The upper lobe showed the same characters, and fluid could be expressed from its substance.

The edema in this lobe was more marked than in the other lobes or in the other lung. The bronchi showed much frothy fluid but their lining was not congested.

*Heart.*—Weight 415 gm. The heart contained a large quantity of soft chicken fat clot of a more gray color than that usually seen. This clot was present in all chambers of the heart. The heart valves had very little noticeable change. The mitral valve showed a slight amount of fibrous thickening. Scattered over the surface of the heart were petechial hemorrhages. Shining through the pericardium, over the ventricles, one could distinctly discern yellow fatty stippling in the heart muscle. When the heart was opened this stippling was beautifully seen shining through the endocardium, particularly in the papillary muscles and columnæ carneæ. The myocardium was pale yellow in color. On section through the myocardium of the left ventricle the diffuse yellow stippling was well brought out. The muscle was of fair consistency. The F. O. was closed. There was no evidence of fatty change in the endocardium, but petechial hemorrhages were particularly noticeable under and in the endocardium of the auricles. The coronary arteries showed patent lumina with no diminution in size or change in the lining. The aorta was thin and elastic, and showed but little yellow intimal change.

*Abdomen.*—The fatty layer was well marked and of a pale yellow color. The recti muscles were bright red and quite thick. The great omentum was fatty and covered the upper coils of the small intestine. The intestines were somewhat collapsed. There was no fluid in the abdominal cavity. The appendix was small showing a slight constriction about the middle due to a band of fibrous adhesions. The organ lay behind the lower end of the cecum and pointed upwards. The mesenteric glands were not enlarged. Some were slightly reddened, suggestive of the hemolymph gland type. Throughout the mesentery were many tiny petechial hemorrhages. The liver reached to the costal margin. The diaphragm arched to the right 5th rib and 5th space on the left.

*Stomach.*—The stomach was of good size. There was a pale smooth healthy looking lining. The pylorus was small and normal.

*Intestines.*—The small intestine showed nothing of note and was healthy. The lymphoid follicles were not enlarged. There was a good deal of sacculation of the wall of the large bowel, but no diverticula were noted.

*Liver.*—Weight 1825 gm. Measured 29x17x9 cm. The margin was sharp. The surface was smooth and shiny. Through it yellowish stippling could be seen. On section the liver was of a chocolate color, swollen and glassy. The outlines of the lobules were not distinct. Scattered thickly over the cut surface of the liver was a fine pinhead sized, yellowish white stippling. These areas were not regular in size. They appeared to lie in the outer portion of the lobule. The whole cut surface was somewhat nutmeg in appearance, although no central zone of congestion in the liver lobule was present. The liver surface was more brownish than the typical nutmeg type. In consistency the liver was about normal. The gall bladder was small and contained about 50 c.c. of tarry bile. The wall was thin and pigmented by bile on its inner surface.

*Pancreas.*—Measured 19x3x2 cm. The organ was firm. The cut surface showed grayish lobules which were normal.

*Spleen.*—Weight 190 gm. Measured 13x8.5x3.5 cm. The notches were quite distinct and the capsule appeared a little wrinkled and was covered by a geographical mottling of white lines suggesting lymphatic vessels. On section through the spleen, the organ appeared fairly firm, and little substance could be scraped off with the knife. The cut surface showed very prominent Malpighian tufts. The trabeculae were but little evident. At two points a slightly darker spleen substance was observed. These areas were roughly triangular, but not clearly outlined. They were not suggestive of true infarction. In size these two areas were 1 cm. across the base and 1.5 cm. in depth.

*Left Kidney.*—Weight 235 gm. Measured 12x7x5 cm. The capsule peeled readily leaving a slightly granular and extremely pale cortex. In the capsule there were a few petechial hemorrhages. On the cortex there was a stippling of congested vessels. Scattered over the surface of the cortex were a number (about 12) of round slightly raised bodies 2 mm. in diameter. On section through these bodies they were seen to extend into the cortex about 2 mm. They had a light green color, were perfectly smooth, clearly outlined, and of the consistency of the cortex of the kidney. On section through the kidney the cut surface presented marked pallor, decided swelling of the cortex, and an indefinite marking. The glomeruli were poorly shown. In the deep cortex several green nodules similar to those described on the surface were to be seen. There was also some diffuse, whitish streaking and stippling which gave the cortex a rather granular appearance. The

fine capillaries of the cortex stood out prominently. About the pelvis there was considerable fat. The pelvis and ureters were healthy.

*Right Kidney.*—Weight 200 gm. Measured 5x5x3 cm. The capsule peeled fairly readily leaving very pale cortex with fine stippling of congested vessels on the surface. Similar small slightly green oval bodies were noted on the cortex. The cut surface of the kidney showed the same appearance as the left, with pale, swollen cortex and indistinct marking. There was one more decided green nodule in the cortex of the same size as the others. The deep cortex was similarly thickened, showing yellowish streaking and stippling as seen on the opposite side. The pelvis and ureters appeared free. There was a moderate amount of fat about the pelvis.

*Adrenals.*—The adrenals were of good size and showed marked yellow cortical layers and small white medullary portions.

*Bladder.*—The bladder was of good size. The wall was smooth and pale. The ureters were patent. The prostate was small and pale in color. The cut surface was glassy.

*Testicles.*—The testicles were of normal size. The cut surface showed a brown, soft character. The tubules could be easily pulled out.

*Bone Marrow.*—The bone marrow of the femur was pale red in color and appeared very moist. The color was darker and more intense than the normal marrow. In the marrow a large amount of cancellous bone tissue was found.

#### MICROSCOPICAL EXAMINATION.

*Green Tumor Mass in Pleura.*—The section showed a very cellular structure. The stroma was loose and appeared in most places as a fine reticulum. In other places it was denser and showed numerous collagen fibers. The only blood vessels seen in this stroma were capillaries. The predominant cell was rather large, about 12 micra in diameter, and mononuclear. The shape was round or slightly oval. The cytoplasm (stained with eosin and methylene blue) was neutrophilic and contained no specific granules. However, with higher magnification the cytoplasm was seen to have a ground glass appearance. A cell membrane was present. This cell had a large round, oval or sometimes irregular nucleus, vesicular in character, with a definite nuclear membrane and rather scattered, coarse, deeply staining chromatin granules or masses. Exclusive of these scattered chromatin masses, the nucleus was pale. Cells with smaller deeply staining nuclei and a relatively great abundance of neutrophilic cytoplasm were rather common. There was also present a number of polymorphonuclear leucocytes and eosinophilic and basophilic myelocytes. Very few typical lymphocytes were seen. Clear nuclear division was not seen, but binucleated forms were present. One of the sections showed the tumor cells to have entirely surrounded several large nerves. Fat globules lay between the tumor cells in many places. Another section of the tumor showed the cells to have surrounded a spinal ganglion. Some of the cells had invaded the substance of the ganglion.

*Bone Marrow.*—Section of the bone marrow showed a moderate congestion and a slight amount of marrow fat. The bone marrow cells were nearly all of the types described in the section of the tumor. About one per cent of the cells were lymphocytes.

*Lung.*—The section showed a deposit of granular precipitate throughout the alveoli associated with considerable red cell infiltration. Some of the alveoli contained fibrin. The walls of the alveoli were thickened, due to the dilated blood capillaries filled with tumor cells lying in the alveoli and surrounded by masses of fibrin and red blood cells. A moderate number of polymorphonuclear leucocytes were also noted. There was considerable anthracotic pigment about the vessels and bronchi. One of the large pulmonary vessels contained thrombi, made up of tumor cells, blood and fibrin. Occasionally, endothelial cells containing anthracotic granules were seen lying behind the alveoli. The tumor cells seen in the blood vessels, when stained with hematoxylin and eosin, had much the appearance of plasma cells.

*Heart.*—The section showed a rather loose, somewhat edematous interstitial tissue, which caused a moderate separation of the muscle fibers. The fibers were not very clear in their staining. Their transverse striations were not well seen, though the longitudinal ones were much better brought out. The nuclei were clear. There was very little pigment about the nuclei. The muscle fibers further had a finely granular, vacuolated appearance. There was no cellular infiltration present, but in the capillaries an unusual number of cells were seen which were similar to those seen in the blood smears.

*Liver.*—Section of the liver showed some congestion. The general outline of the

liver lobules was a little altered by the presence of large areas of necrosis. These necroses lay mostly in the central zone, although many reached into the more peripheral parts of the lobule. In the necrotic areas, there was an infiltration of cells similar to the type of cells seen in the tumor. A few polymorphonuclear leucocytic cells were seen. An infiltration of the tumor cells was also seen around the portal systems. Under high magnification, the indistinct shells of the liver cells could be still made out. In other portions of the section, liver cells of the central zone appeared very watery, showing but a cell membrane and nucleus. The rest of the liver tissue, particularly the peripheral zone, was relatively healthy, although the cells were swollen and granular. Much bile pigment was present in the liver cells throughout. The sinusoids were dilated and filled with granular precipitate. The bile capillaries were also dilated. In the sinusoids were many tumor cell elements of the usual type. Lymphoid cells were also seen in moderate numbers about the portal systems. Fat vacuoles were numerous in the central zones of the lobules.

*Spleen.*—The section of the spleen showed no congestion. The follicles were clear in outline. The trabeculae were not large or numerous. In some of the follicles, masses of hyaline were to be observed. The sinuses were packed with mononuclear cells, similar to those of the green tumor mass. Through the section much blood pigment was noted within the endothelial cells. A hyaline change was observed in the walls of many of the vessels. The pulp was filled with cells similar in type to those found in the blood stream elsewhere, and also to those noted in the tumor mass. The polymorphonuclear leucocytes were few in number. The stroma in the spleen was not increased. In one portion of another section of the spleen was seen a large area surrounded by fibrous tissue. In the center of this area, there was a homogeneous pale blue staining material. Surrounding the area of fibrosis, there was a rather thick wall of tumor cells.

*Kidney.*—The section of the kidney was taken through one of the green nodules. There was also a good deal of uninvolved kidney tissue present in the section. The tubules showed granular looking cells with a moderate amount of debris in the lumen. The cells were quite irregular in outline and some were devoid of nuclei. The glomeruli were not changed, except in their capillaries, where a few abnormal cells were seen. In the capillaries of the interstitial tissue, tumor cells were numerous. The green nodule was seen to be a mass of tumor cells, which, in the center, had entirely replaced the tubular structure, while towards the periphery the necrotic remains of tubules were still to be seen. Near the outer margin of the tumor mass, the tubules were separated, but still living. The outline of the tumor nodule was fairly regular and quite devoid of any capsule, while the kidney tissue adjacent appeared normal. The cells of the nodule were the same as those noted in the tumor elsewhere. Another section of the kidney showed a little connective tissue increase following the course of the blood vessels. An occasional glomerulus was fibrosed.

Sections of the pancreas, prostate and adrenal showed normal characters.

*Postmortem Bacteriology.*—Cultures of the heart blood showed streptococcus pyogenes.

*Oxydase Reaction.*—Examination for oxydase granules by the Schultz method was made with the tissues one year after death. No oxydase granules were seen by this method due to the age of the materials. However, with Graham's method, which will stain the granules after a long period of time has elapsed, the majority of the tumor cells gave a positive oxydase reaction.

*Anatomical Diagnosis.*—Chloroma; myeloblastic leukemia; hyperplasia of bone marrow; chloroma of ribs, vertebrae, pleura and kidney; fatty degeneration of heart and liver; old bilateral pleural adhesions; petechial hemorrhages of skin, pleura, pericardium, endocardium, heart, mesentery, and lung; fibrosis of lung (right apex); anthracosis of lung; central necrosis of liver; edema of liver; enlarged spleen (slight).

*Resume of Case.*—The patient was a man of 36 years, complaining of weakness and pain in the chest. He had no exophthalmos, or glandular enlargement. Repeated examination of the blood showed a progressive leukemia. Clinical diagnosis was myeloblastic leukemia. At autopsy there was found a greenish tumor mass attached to the inner surface of the ribs and bodies of the vertebrae which when peeled out left evidence of erosion of the underlying bone. Green nodules were found in the cortex of the kidney. The bone marrow of the femur was hyperplastic. Petechial hemorrhages were present in the skin over the arms, thorax, and abdomen, beneath the pericardium and endocardium, in the lung, mesentery, and kidney capsule. The predominant cells of the blood were similar to those of the bone marrow and of the green tumors. Oxydase granules were present in these predominant cells showing them to be of myelogenous origin.



## DISCUSSION.

The study of this case contributes nothing to the etiology of the condition. As with other tumors, nothing is known of the causative factor. The presence of streptococcus pyogenes in the heart blood at autopsy is indicative of a terminal infection coincident with the low bodily resistance present just before death and has no etiological bearing. Sternberg cites eleven cases of acute myeloblastic leukemia, nine of which showed organisms either in the spleen, heart blood, or bone marrow. Seven of these cases showed streptococci, six times alone and once combined with staphylococcus aureus; one showed staphylococcus aureus alone and one staphylococcus aureus with streptococcus and influenza bacilli. He concluded that leukemic blood could be obtained in various diseases and that many cases which had been diagnosed as leukemia were but the reactions of the blood-forming organs to the original infections, and that the above cases of "so-called acute myelogenous leukemia" were nothing other than acute general infections which proceeded with myelogenous and myeloid metaplasia and were to be differentiated from the true and genuine leukemias. He offered this in explanation why some of the so-called "acute leukemias" can be cured while the true leukemias always have a fatal ending. But when it is considered that Strauch, in a series of two thousand postmortem blood cultures found an organism in 50.1 per cent of the cases and Fredette in one hundred and nineteen blood cultures taken immediately after death found organisms in one-third of the cases, it is quite probable that some of Sternberg's infections were terminal ones and were not the cause of the leukemic blood pictures. Rother found the same blood picture in a case of acute miliary tuberculosis as in myelogenous leukemia, but there is no way by which we can exclude a myelogenous leukemia as being coexistent with the acute miliary tuberculosis. Gans also recognized a similarity sometimes existing between the blood picture caused by infectious diseases and the blood picture present in spontaneous disease of the myelogenous system.

Although the case under discussion occurred in an adult, the great majority of cases are seen in children. This is sometimes explained as due to the fact, that in childhood there exists a relatively greater amount of red marrow and the hematopoietic organs are more active. But why should not myelogenous leukemia also occur more often in children, if this is the cause? The cell in chloroma is often more embryonal than that found in the ordinary type of acute leukemia. This, and the fact that the cell appears more malignant than that of acute leukemia may have something to do with its occurrence in children.

*Color of Chloroma.*—The greenish color of the chloromatous mass over the ribs and vertebrae faded rapidly on exposure to light and air. Portions of this mass kept in formalin regained its greenish color upon the following day, when immersed in hydrogen peroxide solution. After a period of one year in formalin, the tissue did not regain its former color after a like treatment. The same occurred with regard to the color of the green nodules of the kidney. The green color did not go into solution in either the formalin or the Zenker's solutions in which tissue was preserved. Nearly all observers noted the rapid fading of the color on exposure to light and air, and that the color cannot be preserved by means of chemical solutions, nor can it be recovered by ordinary

aqueous solvents. Exceptions, however, are found to these usual findings. Dock and Warthin in their case (1904) noticed a temporary increase in the depth of color after the removal of the tissue. Alt found that the tissues in his case retained their dark green color in formol solution and the fluid itself was a dirty green color. Risel reported that the color was not preserved in Kaiserling's solution. Burgess observed that tissue preserved in 10 per cent formalin for three years regained its former color in hydrogen peroxide and that in one instance the color was preserved several weeks in a strong solution of sodium bicarbonate, and to a very slight degree in certain of the tissues fixed in formalin and in Kaiserling's solution. Potassium hydroxide restored the color slightly to formalin treated tissue. Walls and Goldsmith say that the color in their case persisted in Kaiserling's solution for four and one-half years. Ayers found that on placing the green tumor in alcohol, the color disappeared entirely within twenty-four hours, but a specimen kept within a corked bottle retained its greenish color. The bone marrow of the tibia in this case showed a redder marrow than is usually present. The character of the tissue of the ribs and vertebræ, where one would expect more change present, was not examined. Burgess thought the redness of the bone marrow in his case was due to congestion of the capillaries. About seven cases have been reported which showed green marrow in some of the bones.

Various theories have been offered for the green color. The variety of the color reactions reported has led some observers to the opinion that it is not always due to the same pigment. By some it has been attributed to iron or fatty substances, but neither of these has been demonstrated as the cause. Reynolds held that the greenish color was of the nature of a fatty acid combined with iron. Walls and Goldsmith concluded that the color was not due to a foreign pigment such as iron, and believed that the consensus of opinion indicates that it is something inherent in the cells of the tumor masses, much the same as the chlorophyll of green plants. Schmidt suggests that the color might be due to the shape of the cells. Risel, Lubarsch and Weinberger demonstrated that the color was not of bacterial origin. Treadgold found that the green color is not present from the beginning, but that it makes its first appearance in the early lesions, and, therefore, that cellular degeneration plays a part in the formation. This degeneration may be due to the age of growth or facilitated by the toxemia. He suggests that possibly a degeneration of the granules or the perigranular protoplasm of these cells, or an abortive attempt to form granules, is the real source of the color, aided by broken down products of hemoglobin.

Sections of the green mass were stained for hemosiderin by Nishimura's modification of the Perls reaction and for fat by Sudan III, but neither were found to be present in the chloroma cells. Risel demonstrated hemosiderin granules in the tumor cells, but did not ascribe the color to these. Lubarsch was unable to find pigment granules in either sections or fresh preparations. Dock (1893) noticed abundant highly refractile granules in the cells of frozen sections. Huber and Chiari found granules which they thought were fatty in nature. Von Recklinghausen believed the color to be of a parenchymatous nature similar to the color of old pus. In the case of Dock and Warthin (1904) no pigment granules were present. Osmic acid and Sudan III tests were nega-

tive. That the color is not a postmortem finding is proved by the green lesions noted during life. Dock suggested that "the peculiar color was simply an exaggeration of the greenish tint so common in the blood clots and neoplasms of leukemia."

At the suggestion of Dr. W. W. G. MacLachlan, examination was made of the white blood cells from a case of myelogenous leukemia at the Mercy Hospital. The leucocytic count was 1,000,000 per cubic mm. Over ninety-nine per cent of these cells showed oxydase granules. Blood was drawn into a test tube with sufficient sodium citrate solution to prevent clotting, and the test tube was corked and allowed to stand twenty-four hours. When examined the buffy coat of white blood cells had a yellow color with a faint greenish tint. After standing for five days in the ice box, this layer showed a decided greenish color and the serum also had a slight greenish tinge. Upon shaking the tube and mixing the red blood cells and the leucocytes, the greenish color was entirely obliterated by the red. The formation of this distinct greenish color in the leucocytes on standing five days, emphasizes Treadgold's theory that the color is not present from the beginning, but that cellular degeneration plays a part. If this greenish color has any relation to that of chloroma, one might expect it where myelocytes and myeloblasts are present *en masse*, as in the cellular tumors of chloroma, which has but little stroma and is practically devoid of red blood cells. Chloroma would then be a type of myelogenous leukemia where the myelogenous cells appeared in dense collections. However, the green color observed in the myelocytes of the blood did not fade on exposure, nor did it deepen on addition of oxidizing reagents. That these two greenish colors may have a relation seems possible, but evidently they are not identical. A similar examination was made of the blood from a case of lymphatic leukemia where ninety-five per cent of the cells were lymphocytes. No green color whatever developed in the layer of white blood cells in this case.

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## THE THYMUS—A SUMMARY\*

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AT an early stage of development of the human embryo, a series of pouches (branchial) are formed in connection with the pharynx. These all resemble one another except as regards their size. The anterior are larger, and from them the primitive tympanic cavity and the palatine tonsils arise. The posterior (the third and fourth) give rise to the thymus and the parathyroids.

The thymus in an early stage is a paired organ of epithelial origin, and in young fetuses (50 mm.) it appears as a closed vesicle lined with high cubical epithelium. During later growth and differentiation the small cells and cell nests appear, and cortex and medulla are formed. In its mature state the thymus has taken on a distinct lymphoid appearance, its masses of cells of lymphoid character enclosing varying numbers of cell nests which are known as Hassall's corpuscles. These are somewhat similar, in appearance, to epithelial pearls, which are the result of the concentric, overlapping arrangement of the flattened epithelial cells.

That these corpuscular cells are truly epithelial there seems to be no doubt, but there is no general unanimity concerning the other, so-called thymus, cells. By some they are presumed to be immigrated cells (thymus lymphocytes); by others, more especially by Stöhr,<sup>1</sup> they are looked upon as modified epithelial cells. Comparative and theoretic considerations undoubtedly speak in favor of their epithelial nature.<sup>2</sup> If this be true, that the thymus is permanently a true epithelial organ, then the typical tumors which originate in the thymus are indeed anomalous, for they have distinctly sarcomatous characteristics. Yet there are certain biologic facts that seem to speak for the lymphoid nature of the thymus cells. One of the effects of the action of the roentgen rays upon the thymus is disintegration of the lymphocyte-like cells of the organ. During this process, lymphocytolytic substances are produced which, if injected into other animals (peritoneally), result in lysis of the lymphocytes in the appendix and mesenteric lymph nodes.<sup>3</sup>

The thymus, beginning its active growth before birth, continues growing for some time—up to the time of puberty, it is said—after which period involution occurs. Its period of growth ends therefore with the beginnings of the ascendancy of the sexual glands. There is here the suggestion of a certain antagonism between the two sets of glands. But for one reason or another, involution may be hastened or delayed. It may be hastened by bad conditions of health, and delayed or slowed by good conditions. Severe illness, especially the infections of childhood, are apt to be succeeded by precocious involution.<sup>4</sup> This apparent relationship between thymus physiology and the general state of the organism led Sahli to speak of the gland as the measure of the state of nutrition. With precocious involution of the thymus there seems to be no precocious development of the sexual glands—nor the reverse.

The thymus is believed by many to be an important internal secretory or-

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gan, which exercises important powers over growth. It is possible that it is able to stimulate growth even though it seems to lack the power of exciting differentiation. Gudernatsch's<sup>5</sup> work indicates that it may be able to suppress differentiation. It is believed that it exerts an inhibitory action upon the sexual glands, the adrenals (especially the cortex), the thyroid, spleen, and pancreas, but these actions are not clearly demonstrated, and seem rather to be based upon coincidences than upon exact experimental results. Changes in the thymus are associated with such syndromes as status thymico-lymphaticus, exophthalmic goiter, dwarfism, achondroplasia, acromegaly, and certain types of precocious adipose states. As a matter of fact, variations in the size and microscopic appearance of the thymus are associated with so many morbid states, and so little has been accomplished by experimental work on it, that very little can be said definitely regarding its physiologic relationships. Our own experience is that there are no definite lesions or changes in the thymus that are associated with other organic or general physiologic abnormalities. This, it would seem, is a minority report. Consensus of opinion is in the opposite direction.

Since the first attempt at thymectomy by Restelli, in 1845, and the publication of Friedleben's experiments, in 1858, a large number of workers have studied the behavior of animals after incomplete and complete operative removal of the thymus. For the most part, until very recent times, all have reported that extirpation of this gland has no more than indefinite and transient after-effects. In 1898, Calzolari noted that the thymus undergoes hypertrophy after castration (rabbits), and J. Henderson called attention to the fact that in cattle castration is followed by increased growth and delayed thymus involution. Noel Paton, using guinea pigs, confirmed Henderson's observations, and added that if thymectomy is done before puberty, the testicles undergo unusually rapid evolution. It has also been remarked that the thymus is larger in capons than in cocks. Additional evidence of interrelationships between other glands and the thymus are offered in the observations of Tandler and Gross, that the latter gland is abnormally persistent in eunuchs; that splenectomy is followed by hypertrophy of the thymus (Mattei); that hyperplasia of the pancreas is apt to follow thymectomy (Mattei); that the thymus is commonly large in hypopituitarism; that it is increased in size after hypophysectomy (Cushing); that persistence of the thymus is fairly constant in acromegaly; that hypertrophy of the adrenals, both cortex and medulla, follows thymectomy (Klose, Mattei, Halsted); that excision of the adrenals is followed by hyperplasia of the thymus; that the use of adrenal extracts produces thymus involution (Wasterson, Boignet); that after thyroidectomy the thymus atrophies; and that after thymectomy, there is an increase of thyroid activity.<sup>6</sup> These data seem to suggest that the thymus is antagonistic to most every other ductless gland, which seems unreasonable. It is supposed that when these changes do not occur it is because accessory thymus tissue exists.

The most striking results of extirpation of the thymus have been obtained by using young animals. After the age of puberty, removal produces less obvious change. The reports chronicle very remarkable skeletal effects, largely in the direction of delayed development. Appearances suggestive of those of spontaneous rickets were reported by Basch,<sup>7</sup> who used young puppies in his

experiments. He noticed osseous changes, beginning two to three weeks after operation, which seemed to indicate deficient or delayed ossification, resulting in increased ease of fracture and increased flexibility. At points of fracture, the callus was deficient in lime salts. With these changes there were also nervous manifestations, some of which were peripheral (hyperirritability) and some central (decreased intelligence). Klose<sup>8</sup> practically duplicated Basch's experiments but used very young puppies (10 to 20 days old), and watched them for longer periods. He observed that after a period of about two weeks, the animals showed increasing adiposity, polyphagia, and loss of muscular activity, following which stage came a third, during which the animals lost weight rapidly, and also lost muscular and bony strength, but retained their appetites. At the same time they lost all psychic power, and finally, in the course of 3 to 14 months, died in coma. The bones from such animals showed extreme modifications which are characteristic of osteoporosis, and which included cyst formation, irregular thickening of the epiphyseal cartilages and deficient linear growths. Fractures which were more easily than normally produced, were healed by fibrous union. The bones showed a loss of about 35 per cent of calcium salts. Klose's explanation is that thymus removal takes away the locus of nuclein synthesis, and that this results in an acidosis (nucleic acid) which causes the removal of lime salts from the bones and at the same time produces an edema of the brain. He also assumes that the spleen assumes the thymic function after involution of the latter, a conception which is supported by the association of hypertrophy of the two organs in status thymico-lymphaticus and by the fact that x-ray, allowed to act upon the thymus alone, leads to a decrease in size of thymus and spleen.<sup>9</sup> The work of Klose, Klose and Vogt, Mattei, and others, points then to the fact that thymus removal produces important general changes in metabolism. Growth is delayed, ossification is slowed, the bones become soft and easily fractured, and healing of fractures takes place slowly and frequently by fibrous union. Also associated with these skeletal changes, a cachexia thymopriva develops after an uncertain latent period and is characterized by lassitude, muscular weakness, emaciation and increased stimulability of the peripheral nerves. Criticism of certain interpretations of the results following thymectomy are based upon the possibility of the removal of parathyroids with the thymus.

Pappenheimer's<sup>10</sup> observations have led him to believe that while rachitic changes do occur in white rats (with which he worked), these are not due to thymectomy, but to spontaneous disease. He was not able to confirm the results of Klose and Magnini. Nordmann<sup>11</sup> believes that the thymus is not an essential organ, and that its removal in young dogs has no important effects, although Flesch<sup>12</sup> has produced evidence that the organ is essential to the life of young rats.

The whole outcome of the work up to the present time seems to be as Wiesel says, that our knowledge of the function of the thymus and of its relationships to other glands is still deficient. One may be permitted to believe that the thymus may be a ductless gland which has a close relation to bone metabolism, to the chromaffin tissue and to the sexual glands.

The thymus is implicated in three well isolated clinical entities beside rachi-

tis; namely, status thymico-lymphaticus (*mors thymica*), exophthalmic goiter, and Addison's disease. Changes in the gland are also associated with various infections, certain cachectic states, with states of precocious adiposity, with dwarfism, and with certain forms of achondroplasia. In acromegaly it is fairly constantly persistent. The relationships which exist between the thymus and other glands is variously interpreted. Hammer divides all the ductless glands into two groups according to their action as excitors or depressors of the thymus. Others see little evidence of definite relationships.

#### STATUS THYMICO-LYMPHATICUS.

It has been noticed from time to time, especially since the reports of Paltauf, that apparently healthy individuals, who have died suddenly and unaccountably during anesthesia, or after falling into the water, or after apparently unimportant traumata, have possessed an enlarged, perhaps hyperplastic thymus. The suspected relations between the enlarged gland and death is expressed in the term *mors thymica*. It has also been noticed that, in these cases, the lymphoid tissue generally is hyperplastic. In typical cases, not only the peripheral lymph glands are large, but the whole lymphoid tissue of the intestinal tract as well appears remarkably hyperplastic. The spleen also is enlarged. Hence the term lymphatism, or status lymphaticus. In some cases only the lymphoid tissue is hyperplastic; in other cases, the thymus alone is affected; often both. In many such cases there is, beside the glandular condition, also evidence of defective physical development, for which condition, in prethymic times, the expression lymphatic disposition or temperament was used. Typical evidences of outspoken physical incompleteness are shown in a certain infantile appearance characterized by obesity, infantile genitals, and defective growth of the hair, especially the pubic.

Not uncommonly the cause of death in status thymico-lymphaticus is believed to be due to the enlarged thymus, almost filling the upper opening of the thorax, resting upon the great vessels, and the cardiac auricles; and hemmed in by the manubrium sterni and the upper ribs, which becomes acutely congested and compresses the organs about it (including the trachea), and so causes rapid death. In less extreme circumstances it leads to dyspnea which disappears when the organ becomes less swollen or after it undergoes involution. It is known that the x-ray is able to cause atrophy of the thymus and that it is very effective in preventing attacks of thymic asthma, as has been shown by Friedlander. The facts that x-ray treatments reduce the size of the thymus and that the effects of the treatments are very prompt, indicate that there is at least some basis in the physical cause of *mors thymica*; and, moreover, if the latent period that exists between operations and symptoms be actually due to the presence of thymus substances in the body, then it would seem that there should also be a very distinct latent period between x-ray exposure and relief which does not seem to be true.

It has also been assumed that the cause of the complex status thymico-lymphaticus is an overfunctioning of the gland, and that under these circumstances there is an excessive amount of secretion given to the system. There seems to be as little proof of this as one might expect from our knowledge of

the physiology of the gland. N. Paton believes that the syndrome is essentially a pluriglandular one in which several glands participate, and that in the absence of the secretions of these glands and because of general imperfect development, death is more easily produced by traumata which would otherwise be ineffective. Wiesel also considers the complex the expression of a general constitutional anomaly—a general tissue weakness. This generally anomalous condition is suggested by the frequency of abnormalities in persons, the subjects of status lymphaticus. The more frequent lesions found in connection with it are hypoplasia of the adrenal medulla, narrowing of the aorta, congenital heart lesions, enlargement of the thyroid, and of the spleen and lymph glands. Also, in children, these lesions are apt to be the lesions of rickets plus a large thymus.

It has been said that primary and secondary morbus Addisoni are almost regularly associated with status thymico-lymphaticus.<sup>13</sup> It seems entirely possible that this is true of the Addisonian complex when the chromaffin tissue is generally damaged, for the occurrence of chromaffin hypoplasia with thymus hyperplasia seems to be a fact, but whether this is so true of the purely secondary types is a question, though Bartel observed that the adrenals were not infrequently found to be tuberculous in status lymphaticus. In one case in the Cincinnati Hospital, in which the symptoms of a developing Addison's disease were present, and in which there were tuberculous lesions in one adrenal, there was no evidence of any thymus tissue, even in microscopic sections. This case was a distinctly secondary one, in which there were progressive pulmonary tuberculous lesions. There were also no discoverable lesions in any of the ductless glands examined (pancreas, pituitary, pineal, thymus). The chromaffin tissue of the adrenals was hypoplastic.

More striking, however, is the appearance of enlarged thymus in Graves' disease. It was long after von Miculicz had remarked upon the presence of an enlarged thymus in exophthalmic goiter, and long after Rehn had suggested operative attentions for the thymus in such cases, that Garré and Capelle reported their striking observation that in 95 per cent of fatal cases of Graves' disease an enlarged thymus was present. Since that time the records have multiplied, and now there seems no doubt that a hyperplastic thymus is a very important factor in the disease. Hart goes so far as to state his belief that there is a pure thymogenic form of the exophthalmic goiter. Garré has said that the blood picture of Graves' disease returns to normal after removal of the thymus; that intraperitoneal injections of thymus extracts produce the blood picture which Kocher believed characteristic of Graves' disease; and that the thyroids show evidence of regressive changes after thymectomy. Gabele has said that implantation of normal thymus in thyroidectomized dogs prevents the appearance of cachexia thyropriva, and Bircher has been able to produce a typical Graves' disease by intraperitoneal implantation of fresh pathologically hyperplastic thymus. The recent observations of von Haberer, Klose and others tend to support the conception of the dual nature of Graves' disease, and the experiences of Halsted<sup>14</sup> tend in the same direction, although von Haberer<sup>15</sup> says, in agreement with Kocher, that cases of Graves' disease without thymus hyperplasia occur, but he also says that cases of hyperplastic thyroid in association with enlarged thymus glands exist without symptoms of exophthalmic goiter.



There still remains the association between thymus persistence and hyperplasia, and dwarfism, achondroplasia, and other abnormal states. Whether these are constant associations and active ones, we cannot tell—the answer must be left to the future to decide. All that seems at all definite is that the thymus seems to be a factor—to play a part—in the processes of growth and, as Romeis<sup>16</sup> says, the younger the animal, the surer is the growth quickening action of the gland. His experiments gave somewhat the same results as those of Gudernatsch.

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## ORTHOSTATIC ALBUMINURIA\*

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THE following is a brief clinical note concerning a case of albuminuria of the orthostatic type. The justification for presenting it rests in part upon its apparent etiology and in part upon its yielding, to a great extent at least, to surgical intervention.

Observers are not entirely in accord as to the conditions underlying orthostatic, or following Heubner,<sup>1</sup> philologically more correct, orthotic albuminuria. The experimental work of Jehle<sup>2</sup> speaks convincingly for the rôle of a lumbar lordosis in its causation, and this theory has perhaps the greatest number of adherents. Pollitzer<sup>3</sup> emphasizes the importance of a congenitally insufficient faucial lymphatic ring, pointing to the frequent association of angina with this form of albuminuria. Jehle, while he concedes the truth of this last fact, states that disappearance of the albuminuria, following the removal of the offending tonsils, has never been demonstrated. This assertion was made by Jehle in 1913, in a comprehensive monograph<sup>4</sup> on the subject.

Among other causes suggested in explanation of this phenomenon are the following: A persistently low pulse pressure in the renal glomeruli (Erlanger and Hooker<sup>5</sup>); Teissier's<sup>6</sup> view that it is a manifestation of a latent tuberculous infection—the so-called pretuberculous stage; that it is due to increased renal permeability, hence its frequent occurrence in families (Leube<sup>7</sup>); that it is the sequel of some infectious process; that it is an evidence, purely, of a labile vasomotor system; and, finally, that it is the result of some interference with the extrinsic renal vessels, such, for example, as would occur when a full colon caused pressure upon the renal veins when the individual assumed the erect position.

Certain predisposing factors are better known. Thus, the condition is generally considered as distinctly more common in boys than in girls, though with this Heubner<sup>8</sup> and Langstein<sup>9</sup> disagree. Furthermore, it is essentially a manifestation of adolescence. Schaps,<sup>10</sup> in the Berlin clinic, found 94.12 per cent of his cases between the ages of five and fifteen years. Heubner's statistics are not entirely in harmony with those of Schaps as he classifies 38 per cent of his cases between the ages of sixteen and twenty years, and 17.86 per cent between the ages of twenty-one and thirty. Finally, as shown especially by English sources, it is of very frequent occurrence.

The essentials of the case forming the basis of this report are as follows: The patient, a young medical student age twenty-one, consulted the writer after discovering in one of his laboratory courses that his urine contained albumin in large amount. The tests employed were the heat, the Heller, and the saturated sodium chlorid-acetic acid. The quantitative determination by Esbach showed three parts pro mille. This discovery was a great surprise to the young man as his general health had never been better.

As to the anamnesis, it was brought out that aside from frequent attacks of

\*Read before the Clinical Society of the Michael Reese Hospital, Chicago, April 3, 1916.

tonsillitis, he had always been well. Incidentally, it is perhaps worthy of note that a brother had had a nephritis following scarlatina and that his father had succumbed to chronic Bright's disease. The mother is subject to recurrent sore throat.

Examination showed a stout and rather muscular individual 5 ft. 11½ in. in height, weighing 217 pounds. There was no lumbar lordosis. The tonsils were large and hyperemic, and rather deeply buried; and some of the tonsillar crypts contained plugs. There was no cervical adenopathy. The lungs were negative. The heart seemed somewhat enlarged, as determined by the ordinary methods, the left border being in the mammillary line. Fluoroscopically, with the narrowed screen, MR measured 4.7 cm. and ML 9.3 cm., making a transverse diameter of 14 cm., which is the upper limit of the normal for a male individual of his age, weight and height according to the tables of Dietlen.<sup>11</sup> There were no auscultatory anomalies.

The abdomen was without changes; and ankle edema was absent. The blood-pressure on several examinations was 120 systolic and 80 diastolic. On one occasion the systolic pressure registered 130 mm.

The urine contained albumin by the various tests mentioned above. There was present in addition a body precipitated by acetic acid in the cold. The reaction for this substance was so pronounced as to suggest that the bulk of the material producing the positive albumin tests was of this nature. This acetic acid precipitable substance, furthermore, was present even though the reaction for serum albumin was negative. Urinalysis showed only one other abnormality; viz., the almost constant presence of scattered hyalin casts.

Further study of the case demonstrated the fact that the occurrence of albumin was strictly associated with the assumption of the upright position. If the patient, having emptied his bladder on a given night, urinated into a conveniently placed receptacle the succeeding morning, the specimen contained no albumin, and also no casts. If the young man arose to urinate, or even if he lifted himself in bed to turn on the light, albumin appeared, and as a rule, casts. The amount of albumin varied, diminishing apparently as the day wore on.

In view of the plans of the patient, it was especially imperative to determine, if possible, the underlying cause of the condition. The fact that the albuminuria was postural in nature did not, of course, rule out the possibility of a renal lesion. In the opinion of some observers, Senator<sup>12</sup> and Langstein<sup>13</sup> among others, casts eliminate the diagnosis of a cyclic or orthotic albuminuria. Sahli<sup>14</sup> and Jehle<sup>15</sup> on the contrary do not regard the presence of a few hyalin casts as militating against this diagnosis; and Jehle and others have observed cases exhibiting casts which definitely proved themselves to be of the orthotic type.

According to Langstein, the presence of a body which is precipitated by acetic acid in the cold, regarded by him as nucleo-albumin, but which is probably a chondroitin-sulphuric acid, is not observed in the nephropathies, and is strongly suggestive of the orthotic nature of the condition.

As to the heart, this is to be said: An organ enlarged to the left or to the right or transversely is very frequently observed in undoubted cases of orthotic albuminuria. This is the so-called *cor juvenum* of Krehl and Germain Sec.<sup>16</sup>

In the doubtful case, an enlarged organ is to be regarded as pathological only when conjoined with other evidences of a renal lesion, particularly hypertension, a ringing aortic second sound and abnormal vessels.

The preliminary examination of the patient took place toward the end of November, 1915. In the absence of a satisfactory etiology of the condition, and particularly in view of the uncertainty as to the gravity of the affair, tonsillectomy was advised, primarily because of the evident pathology in these organs. The writer was influenced to a considerable degree also by the studies of Pollitzer relative to the frequent association of his material with recurrent anginas; though as previously mentioned, and emphasized by Jehle, no case was on record in 1913 of the subsidence of an orthotic albuminuria following the removal of the tonsils.

The tonsillectomy was performed during the holidays. Shortly after there occurred an epidemic of infections of the respiratory tract, and to this the patient fell a victim. Complicating his affection was an otitis media and mastoiditis, the latter subsiding, however, without operative interference. During this illness albumin continued to be present in the urine.

With the termination of the middle ear and mastoid infections, examination of the urine under differing postural conditions was resumed. The results of these later tests, covering a period of six weeks, can be summarized in a few words: The urine, except on one occasion, has been, and continues to be, free from albumin and other abnormal constituents. The exception occurred when the patient, in an effort to subject himself to an unusually severe test, raced up five flights of stairs. The urine collected after this exertion contained albumin by the various tests, though in this particular instance it would be difficult to say whether the albuminuria was of the type under discussion or was merely that seen so frequently after severe exertion. No casts were found.

If one is of the opinion of Jehle that a lumbar lordosis is an essential feature of all true cases of orthotic albuminuria, then the case in question cannot be regarded in this category. If, on the contrary, a lordosis is only one of several factors responsible for the condition, among which is to be emphasized infections of the faucial tonsils, then the case under discussion may be said properly to fall in this group of albuminurias, especially as the presence of such features as casts and an enlarged heart may be explained on satisfactory grounds other than the assumption of a nephropathy.

*Note* (February, 1917—nearly a year since the preparation of this paper).—For several months after the above noted exceptional finding of albumin, a trace of the latter was found in the urine on isolated occasions, particularly during the middle of the day. In the past seven months, however, albumin has been absent at all times and by all tests.

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## DIASTOLIC PRESSURE IN THE AGED\*

BY L. M. BOWES, M.D., CHICAGO.

A LARGE majority of the diseases of old age are due to changes which take place in the blood vessels, heart, or kidneys, and which usually progress to a fatal termination as a result of cardiac failure, cerebral hemorrhage or nephritis. Therefore, it is of extreme importance that a study be made of the condition of the cardio-vascular-renal system by means of measuring the peripheral resistance. This may be accomplished by observing the diastolic pressure, which is a better index and, as it varies less than the systolic pressure, is of much greater importance.

In order to emphasize this point I wish to report 59 deaths which occurred in old people between the ages of 65 and 95, during the past four years.

Fifteen deaths were due to chronic valvular heart disease, 9 to cerebral hemorrhage, 8 to arterial disease, 6 to nephritis, 7 to cancer, 2 to accident, 5 to pneumonia, 2 to acute enteritis, 1 to diabetes, 1 to tuberculosis, and 3 to chronic bronchitis which, in all probability, were also of cardiac origin. There was a total of 35 deaths due to cardiac or arterial disease.

Nephritis was present in a number of cases but was the immediate cause of death only in six instances.

Four hundred observations were made in a series of 161 patients, all but two of whom were between 65 and 95 years of age. The point at which all sound disappeared was recorded as the diastolic pressure. Eighty-six, or 53 per cent, had diastolic pressures between 75 and 95 mm. Hg. The average diastolic pressure for those between 65 and 69 years of age was 82 mm. Hg. After 69 there was an increase up to the period between 85 and 89 when the diastolic pressure was 90 mm. Hg.

### LOW DIASTOLIC PRESSURE.

There were 43 who had diastolic pressures below 75 mm. Hg. The lowest was 58 mm. and the average low was 67 mm. Hg. Any diastolic pressure below 75 mm. was considered abnormal. Eight of those with a persistent diastolic pressure below 75 mm. were normal in health. The rest were sufferers of myocarditis, rheumatism, acute enteritis, carcinoma, anemia or senile dementia, named in the order of frequency.

\*For an earlier paper on Blood Pressure in the Aged, see this journal, ii, 256.

## HIGH DIASTOLIC PRESSURE.

There were 32 who had persistent diastolic pressures above 95 mm. The highest was 143 and the average 110 mm. Hg. Any diastolic pressure above 95 mm. was considered abnormal.

Fifty per cent of those who had persistent high diastolic pressures presented mental symptoms such as headache, dizziness, lack of memory, confused ideas, hallucinations, cerebral atrophy, senile dementia or cerebral hemorrhage.

Nearly all had enlarged cardiac dullness, with an accentuated second aortic sound and a systolic murmur heard at the apex or at the second intercostal space.

Eight had chronic valvular heart disease, two valvular lesions associated with cerebral hemorrhage, and two valvular heart lesions with nephritis. Two others had suffered cerebral hemorrhages. Several complained of cardiac pain and tingling in the fingers and toes.

## CEREBRAL HEMORRHAGE.

As a general rule a sustained hypertension of both systolic and diastolic pressures diagnosticates cerebral hemorrhage from cerebral embolism. But there is a small group of cases in which neither pressures are high. It is not an uncommon thing to see a fall of systolic and diastolic pressures following a small cerebral hemorrhage. I have seen a drop of 25 mm. in the systolic and 5 mm. Hg. in the diastolic pressures within twenty-four hours following such a hemorrhage.

There may be a temporary rise in case a checking of the hemorrhage occurs only to fall again with a recurrence of the bleeding. These cases can only be diagnosticated from cerebral embolism by following them to autopsy. The hemorrhage is quite small and usually is located in the anterior portion of the brain.

A hemorrhage that is profuse or occurs in the region of the fourth ventricle is followed by a high or increased blood pressure.

There is less danger of a blood vessel breaking when the diastolic pressure is low even if the systolic pressure is high.

The arteries were very rigid in all of the cases of cerebral hemorrhage that had low diastolic and systolic pressures.

## ARTERIOSCLEROSIS.

A great deal depends upon the heart and kidneys whether or not there is an increased diastolic pressure in arteriosclerosis. When it is raised the increase is not in proportion to the systolic which causes a high pulse pressure.

There is a great deal of value to be derived from these high diastolic pressure readings because it is an index to the amount of increased peripheral resistance.

There was a slow heart action in some of the cases of marked arteriosclerosis. A pulse rate of 40 to 50 is not uncommonly associated with a diastolic pressure of 104 mm. Hg. or even higher.

On the other hand, we may not have an increased diastolic pressure. Price<sup>1</sup> explains this by saying that "there is less dilatation and recoil to maintain it."

Cornwall<sup>2</sup> states that the diastolic pressure "is usually raised when the arterial supply of the vital regions is involved, but the elevation does not seem to be so high, as a rule, as in chronic nephritis."

Whenever myocarditis develops or the limit of the cardiac reserve power has been reached we have a fall of blood pressure, both systolic and diastolic, regardless of the condition of the arteries.

#### CHRONIC HEART DISEASE.

The diastolic pressure is generally low in aortic regurgitation. It is impossible, in some cases, to make an accurate reading because a sound can be heard until all pressure in the armlet is removed.

A high diastolic pressure was found in aortic stenosis. But as it was associated with marked arteriosclerosis, it was impossible to determine which influenced the diastolic pressure.

A low diastolic pressure was found in all cases of chronic myocarditis.

A difference of 10 to 20 mm. Hg. between the small and large beats was observed frequently in irregular heart action.

#### CHRONIC NEPHRITIS.

Chronic interstitial nephritis is the usual type of nephritis met with, and it is not common to have an uncomplicated case at this time of life.

Arteriosclerosis, chronic myocarditis and uremia are the usual complications which may modify the blood pressure. But where chronic interstitial nephritis predominates, the diastolic pressure is higher than in any other disease of old age. It is generally between 120 and 150 mm. Hg. and a higher pressure is often seen.

The heart hypertrophies in all of these cases in order to meet the increased peripheral resistance. When the limit of the cardiac reserve power has been reached, the diastolic pressure falls with, but not as rapidly as, the systolic pressure, causing a lower pulse pressure.

This explains why we have so many cases of chronic nephritis with low systolic and diastolic pressures in those past eighty.

#### CHRONIC TOBACCO POISONING.

In looking over the records of fifty who have been hard smokers for forty and even seventy years, I find that the blood pressure was influenced by the associated conditions rather than by the tobacco poisoning.

One woman, aged 79, who has been a chronic smoker for many years and who has marked arteriosclerosis, associated with aortic regurgitation, had a systolic pressure of 170 mm. and a diastolic pressure of 105 mm. Hg. The urine was negative.

Some old men who have been inveterate smokers nearly all of their lives had systolic pressures from 160 to 190 mm. and diastolic pressures in the eighties.

It is to be admitted that chronic tobacco poisoning causes a decreased blood pressure in many, and may in younger men, but I do not believe it to be the general rule in these aged smokers.

## DIASTOLIC INDEX.

Williams and Melvin<sup>3</sup> state that "in doing blood pressure estimations we find it desirable to determine the diastolic index while the armlet pressure is being raised; in some (rare) cases where arm compression leads to a marked rise of blood pressure, the diastolic reading taken with a rising armlet pressure may differ from that obtained during the lowering of the armlet pressure (after determining the systolic index) by as much as 20 mm. This was especially the case where simultaneous estimations were made in the two arms. But a decided effect was sometimes observed when only one was tested. Comparison of the diastolic values with rising and falling armlet pressures may thus give important indications of the liability of the arterial pressure to vary in an abnormal way, when a large artery is closed, as in the process of systolic estimation."

This effect was observed, in a series of cases, in which only one arm was tested. There were several cases of marked arteriosclerosis, aortic and mitral regurgitation and one of aortic stenosis which did not vary in this way. Neither did any case of cerebral hemorrhage, nephritis, diabetes, cancer or chronic myocarditis, although many observations were made and some were made but a short time before death.

A variation was present from 4 mm. found in a case of chronic organic heart disease, to 16 mm., found in a case of general edema (including edema of the lungs). Other conditions showing such variations were bronchial asthma, cardiac asthma, marked flatulence, which interfered greatly with the action of the heart and lungs, and one case where the opposite arm had been amputated.

Some cases of aortic and mitral regurgitation showed this but others did not.

One woman, aged 81, with a slight paralysis as a result of a previous cerebral hemorrhage, had an aortic regurgitation.

The rising diastolic pressure was 100 and the falling diastolic pressure was 96 mm. Hg. This variation did not change in the following observations. Shortly after, decompensation took place resulting in the death of the patient.

A man, aged 83, in whom there was no difference between the rising and falling diastolic pressures, died of cerebral hemorrhage and right hemiplegia.

There has been too little work done to learn just what the diastolic index would indicate. It may prove to be of great value especially in surgery before amputation of a limb.

In conclusion I wish to state that the systolic and especially the diastolic pressures should be studied in the aged to aid in diagnoses and intelligent treatment.

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## SOME RECENT STUDIES IN THE CHEMISTRY OF THE CEREBRO-SPINAL FLUID

BY CHARLES E. KIELY, M.D., CINCINNATI, OHIO.

WESTON<sup>1</sup> has made a very careful research on the sugar content of the blood and of the cerebrospinal fluid in mental diseases, using the technic of Meyer and Bailey.<sup>2</sup> An ample amount of material enhances the value of the work. Twenty cases of dementia precox were examined; ten cases of maniac depressive psychosis; twenty cases of general paralysis; six cases of epileptic psychosis; and nine cases of imbecility. The research leads to the following conclusions:

The ratio of blood to spinal fluid sugar varied from 1 to 1.55 (in epileptics) to 1 to 1.72 (in paretics). In maniac depressive psychosis the average blood sugar content was 0.1215 and the spinal fluid 0.0711. A review of the table shows no constant difference between the manic and the depressed stages. In the epilepsies the figures were respectively 0.1151 and 0.0740; in imbecility, 0.1223 and 0.0723; in paresis 0.1140 and 0.0658; in dementia precox, 0.0995 and 0.0604. This last is a disease in which various endocrine glands have been under suspicion, and the sugar content of the tissues might be an important factor in the discussion were the normal not so unsettled. Weston quotes the figures of various workers compiled by Gettler and Baker.<sup>3</sup> Of thirteen observers the lowest minimum is 0.04 and the highest maximum 0.15,—a variation of almost 400 per cent! Pathological sugar values cannot, of course, be predicated when the normal is so uncertain. The general average of all Weston's cases was 0.1145 for the blood, and 0.0687 for the spinal fluid. The sugar content of the spinal fluid of cases of paresis treated daily with inunctions of mercury did not differ materially from that of untreated cases,—the figures were 0.0725 and 0.0718.

Levinson<sup>4</sup> attempted to investigate the reduction of alkalinity of pathological fluids. His technic and interpretations are open to very serious criticism, as very few of his specimens were examined within a reasonable time after withdrawal. Moreover, variations on standing were not studied in the critical period which would surely be soon after they were taken from the body. To quote verbatim (p. 258): "In order to determine the change in alkalinity going on in the fluid after it had been removed from the body different portions of the same fluid were put into the ice box—some of the tubes being plugged with cotton and some with ordinary corks. As was natural to expect, those fluids exposed to air increased their alkalinity in a short time. Of the fluids contained in corked tubes some showed a marked increase in alkalinity, others showed hardly any increase. Pathological fluids always showed an increased alkalinity on standing." Levinson then tabulates his comparisons. Some fluids were re-examined several months after their withdrawal. Two showed no variation while the remainder ranged from 9 to 40 per cent—averaging 12 per cent.

For this reason the work of Levinson seems vitiated as his tables show that of 55 fluids, 21 were not examined even the same day, and in 27 instances there is no record whatever of the time elapsing between withdrawal and examination. Concerning the relation of alkalinity to dextrose content Levinson says: "The lowest alkalinity obtained in meningitis still showed dextrose to the amount of 0.08 which is a normal amount." Weston's table compiled from six authorities varies from 0.046 to 0.080. The value of Levinson's paper is further impaired by tabulating "Insanity" as a diagnosis in a great many of his mental cases.

A third contribution to neurological chemistry is happily one of accuracy and extreme value. Nuzum and Lecount<sup>5</sup> determined the hydration capacity of the brain in delirium tremens as compared with other pathological conditions and with the normal. Essentially the work is a test of the theory of edema elaborated by Martin Fischer;<sup>6</sup> i.e., that the human tissues, being hydrophilic colloids, absorb and retain water in proportion to their degree of acidosis. In bold outline their thesis is that alcohol which is a narcotic diminishes oxygenation in the cerebral tissues, producing thereby an acidosis so that free water is absorbed and the condition we call edema exists. Nuzum and Lecount offer copious material carefully tabulated, and with ample controls. Their technic was to immerse pieces of brain tissue from cases of alcoholic delirium in distilled water for 48 hours and estimate by weighing the amount of water absorbed. In all cases the time elapsing after death is carefully tabulated. The figures for delirium tremens are compared with those from cases of other kinds, six of whom died suddenly. These last are used as instances in which acidosis could not occur, but one at least is open to criticism as the cause of death is given as asphyxia—a condition in which acidosis should develop very rapidly. The only serious objection to their conclusions—the possibility that they are only registering a postmortem acidosis—is met by control experiments on both normal and alcoholic rabbit brains tested soon after death with results confirmatory of the figures for the human. It is to be noted that in neither the human nor the rabbit tissue was there invariably a gross edema.

Specifically their results were as follows: 40 cases of general disease examined an average of 21.3 hours after death showed an average gain of water, by weight, of 64.5 per cent; 6 cases of sudden death examined an average of 24 hours after death gave the same figure—the consonance supporting these two tables as normal. In support of Fischer's claims it is to be noted that the highest figures were obtained in such cases as theoretically should exhibit acidosis, i.e., morphinism 84 per cent, cerebral hemorrhage 74.3 per cent, cerebral embolism 73.5 per cent, and uremia 71 per cent.

Coming to the principal point of the paper, we find that 20 alcoholic brains examined an average of 21.1 hours after death showed an increase of 70 per cent; brains of five rabbits chronically alcoholic, but not reproducing any picture like a tremulous delirium examined an average of 8 hours after death gave an average of 78.9 per cent; control rabbits gave only 70 per cent.

This article by Nuzum and Lecount is followed immediately by that of Hogan,<sup>7</sup> recording the results of treating delirium tremens as an acute acidosis of the brain. The results are a confirmation of this hypothesis.

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## SPLENOMEGALIA—AUTOPSY REPORT

BY JOHN W. SHUMAN, M.D., SIOUX CITY, IOWA.

THIS autopsy is reported on account of the enormous size and weight of the spleen. The clinical diagnosis was myelogenous leukemia on account of the blood picture, accompanied by marked splenic enlargement. Through the courtesy of Dr. Geo. S. Browning who had treated the case with roentgen therapy I held the postmortem.



Fig. 1.—Right lateral view of spleen, showing measurements  $6 \times 13\frac{1}{2}$  inches and the notch



Fig. 2.—Left lateral view.

Autopsy No. 231, held November 15, 1916, at 8 p.m. upon an emaciated male body, weight 125 pounds, height 5 feet and 10 inches, apparent age 30 years. Rigor mortis slight (death eight hours prior). Usual incision. Subcutaneous fat absent. Muscles pale and flabby, also bloodless. Abdomen filled with embalming fluid. Liver at costal margin.

Liver appendages negative. Small and large intestines distended. Stomach flaccid. Appendix without change.

Spleen greatly enlarged; removed with difficulty on account of many dense adhesions all over it. The upper end of this organ was at the left fifth interspace, while the lower end rested upon the floor of the pelvis. The spleen occupied the whole of the left abdomen.

The stomach opened; the whole of the mucous membrane showed pinhead-sized hemorrhagic spots. The mucous membrane of the lower ileum and that of the colon



Fig. 3.—Cross section through thickest portion.

evidenced the same. Kidneys and other intraabdominal organs evidenced no macroscopic pathological change. Heart and lungs showed no gross pathology.

#### SPLEEN TUMOR.

The appearance of the spleen was a bluish-red with many stellate whitish areas over its whole surface, especially the anterior. It cut with great resistance. The cut surface was unusually dry and the normal acinous picture was obliterated. Many bands of dense scar tissue were visible (Fig. 3). The measurements of the spleen were  $13\frac{1}{2}$  by 6 by  $5\frac{3}{4}$  inches (Figs. 1 and 2). Its weight was 9 pounds.

Sections made and stained by Dr. W. G. Rowley showed myeloid transformation. The entire pulp and follicles were transformed into myelocytes (myeloish).

# A QUANTITATIVE SEPARATION OF HEROIN FROM ORGANS\*

By WM. D. McNALLY, A.B., CHICAGO, ILL.

THE usual methods used for the detection of morphine fail to show the presence of heroin. A number of experiments were performed to determine the reason for certain extracts obtained in the two above cases giving the tests for morphine. To demonstrate that the normal hydrochloric acid of the stomach did not cause a deacetylation of the heroin, I added 2 c.c. of a .2 per cent hydrochloric acid, to five samples of heroin hydrochloride, and 2 c.c. of .46 per cent HCl, to three samples, each containing .1 gram of heroin hydrochloride. The eight samples with two blanks containing the same amount of acid were placed in an incubator at 98.8° F. for two hours. The samples were then titrated with N/20 NaOH. The amount necessary to neutralize the blank was deducted from the cubic centimeters of N/20 NaOH used in titrating the .1 gram sample of heroin hydrochloride, in the .2 per cent and .46 per cent HCl, and the HCl found was equivalent to the acid combined with the heroin. (Diacetylmorphine hydrochloride,  $C_{17}H_{17}(O.C_2H_3O)_2ON.HCl + H_2O = 423.68$ .) The acid ether extracts of the samples titrated failed to give a red color with Million's reagent, showing absence of phenolic hydroxyl group. The alkaloid recovered responded to tests for heroin, giving a melting point of 170° C.

TABLE I.

REAGENT	MORPHINE	MONOACETYLMORPHINE	DIACETYLMORPHINE (HEROIN)
Froehde's	Violet color, fading to bluish green	Violet, changing quickly to a blue, fading to a yellow	Crimson purple, gradually changing to blue
Marquis'	Deep purple	Purple soon fading	Brilliant violet soon fading, addition of water changes to yellow
Mandelin's	Yellow, faint violet turning to slate color.	Pale violet fading to slate color	Pale violet, changing to pink, gradually fading
Iodic Acid	Easily reduced	Easily reduced	Not reduced
Pellagri's*	Violet, emerald green on addition of dilute tincture of iodine	Same as for morphine	Responds same as for morphine
Goldsmith's Test	Negative	Positive	Odor of acetic ester
Denige's Test	Positive	Positive	Positive
Modified**			
Nitric Acid	Orange red changing to yellow	Same color as morphine	Dissolves alkaloid with a yellow color, changing on standing to a greenish blue, fades to yellow. The reaction is hastened by gentle heat
Ferric Chloride	Blue color	No color	No color
Prussian Blue Test†	Deep blue color	Deep blue color	No color, upon standing, a blue will appear

\*From the Cook County Coroner's Laboratory, Chicago.

TABLE I (Cont'd).

REAGENT	MORPHINE	MONOACETYLMORPHINE	DIACETYLMORPHINE (HEROIN)
Urotropin and Sulphuric Acid <sup>††</sup>	Purple	Purple	Golden yellow color, changing to saffron yellow, finally blue
Chloral Hydrate and Sulphuric Acid			Yellow. Codein will give deep green
Platinic Chloride	Amorphous precipitate	Amorphous precipitate	Crystals in form of a burr, from dilutions of 1 to 100. (See Figs. 1 and 2.)
Dichromate and Sulphuric Acid	Greenish gray to dirty green		Flesh color momentarily
Wagner's	Precipitate seen under the microscope	Dilutions 1 to 100,000	Gives a precipitate 1 to 100,000, easily seen under AA objective
Melting Point	230° C.	187° C.	171° C.

\*Pellagri's Reaction: Gazz. Chim. ital., 1877, vii, 297; Witthaus & Becker, 1911, iv, 989.

To the solid substance dissolved in hydrochloric acid, add a few drops of sulphuric acid, Sp. Gr. 1.84, heat to expel the hydrochloric acid, temperature about 110° C. In the presence of morphine, codein, apomorphine, and heroin, the residue is reddish violet. Cool, take up with a few drops of hydrochloric acid (1 to 5 dilution); a bright cherry red color is produced. Neutralize with sodium bicarbonate, added in powdered form. A smoky green is noticed as the point of neutralization is reached. Add a drop of very dilute alcoholic solution of iodine; a brilliant green color is obtained. The pigment is soluble in ether forming a purple solution.

Goldsmith's test, dissolve the alkaloid in alcohol in a small test tube, add a drop of sulphuric acid; gentle heat will give odor of acetic ester.

\*\*Denige's Test: Compt. rend. Acad. d. sc., 1910, cli, 1062; Jour. Am. Med. Assn., Aug. 8, 1914, 513.

To a solution of morphine, add a few mils of H<sub>2</sub>O<sub>2</sub>, and a drop of strong ammonia. The solution is then stirred with a piece of copper wire, the solution becomes deep port wine tint. Dilute to .00002 gram of morphine. If the blue color formed masks the red tint, add a few drops of KCN solution at the end of the reaction.

†Prussian Blue Test: U. S. P., IX, 130.

††Urotropin Test: Allen's Commercial Organic Analysis, 1912, vi, 389.

## EXPERIMENTAL WORK WITH ANIMALS.

LAB. NO.	ANIMAL	WEIGHT IN GRAMS	DOSE PER KILOGRAM
1234	Rabbit	2100	.15
1235	"	1821	.05

Rabbit No. 1235 became drowsy, after two hours appeared normal.

Rabbit No. 1234 after receiving the heroin hydrochloride, stretched out the hind extremities, then the front. The head was pulled back, short convulsive movements were noticed, which resembled those due to strychnine, but external stimuli failed to produce convulsions. The rabbit died in tetanic convulsions, from failure of respiration, five minutes after receiving the heroin hydrochloride.

The analysis of the organs of this rabbit (1234), and the organs of a dog poisoned with heroin hydrochloride are given in Table II.

TABLE II.

ORGAN	LAB. NO. 1234	LAB. NO. 1598
Brain		Trace
Heart and blood		.0120
Lungs and blood		.0072
Stomach and contents	.0369	.0296
Intestines	.0193	.0116
Liver	.0085	.0284
Kidneys		.0162
Bladder and urine	.0006	.0071

The heroin was separated from the animal tissues in the case of No. 1234, by the method given for morphine by Peterson and Haines,<sup>1</sup> the alkaloid giving all of the tests for morphine. The alkaloid from the stomach gave a weak test for the acetyl radical. From analysis of No. 1234, it would appear that a deacetylation had taken place in each of the organs examined. To a sample of 81 grams of fresh liver, heart, and spleen from a rabbit, was added .1 gram of heroin hydrochloride. The sample was kept in an incubator for three hours and the alkaloid separated as in sample No. 1234. The alkaloid separated gave all the tests for morphine.

Three samples of 100 grams each of human liver, containing .005 grams of heroin hydrochloride, were acidified with acetic, tartaric, and sulphuric acids respectively. The first sample was extracted with 50 per cent alcohol, the second with 25 per cent alcohol, and the third with water. The first two samples were carried through a process similar to that used in sample No. 1234, the third was extracted by a method suggested to me by Prof. John Uri Lloyd.<sup>2</sup> The alkaloid extracted from each sample responded to all the tests for morphine. This demonstrated that the method of extraction and separation of the alkaloid was responsible for obtaining morphine.

As a result of the above and other experiments, I present the following method for the extraction of heroin, which entails but little decomposition and loss of the alkaloid. The principle involved is the use of a weak acid, a low temperature, and the precipitation of the alkaloid by a hydrated aluminum silicate (Alcresta).

#### METHOD FOR SEPARATION OF HEROIN.

Extract the alkaloid from the suspected tissue by successive portions of 50 per cent alcohol acidified with a few drops of 10 per cent tartaric acid. Keep the temperature of the samples close to 50° C. Allow the combined extractions to remain in a cool place until the fat separates. The fat is separated in a separatory funnel, washed with acidified water, and the washings added to the main extractions. If the sample is allowed to stand overnight, considerable extraneous material settles to the bottom of the flask and can be removed by filtration, washing the filtrate with 50 per cent alcohol.

The alcoholic extractions are evaporated under diminished pressure, the water bath being kept well under the boiling point. The pasty residue is taken up with a little cold acidified water, and the extraneous matter filtered off. The filtrate is allowed to filter into 95 per cent alcohol; the protein substances (albumin, albumoses, peptones) and other impurities become granular on standing, and are easily filtered off. The alcohol is evaporated off under diminished pressure, until the liquid in the distilling flask amounts to about 20 mls. This residue is taken up with cold water, acidified with tartaric acid, and filtered. To the filtrate is added one gram of "alcresta." The "alcresta" is filtered off and the liquid tested for alkaloid. More of the alcresta is added in case the first portion does not remove all of the alkaloid.

The alcresta containing the alkaloid is transferred to a glass separatory funnel and extracted with successive portions of ammoniated chloroform.

The chloroform is evaporated, leaving the alkaloid free, as a residue. If any fat accompanies the alkaloid, dissolve the residue in a .5 per cent hydrochloric

acid, filter and re-extract. The alkaloid is weighed and after testing for heroin, with the color reactions (Table I), the purity can be further substantiated by volumetric methods, melting point of the double platinum salt, and the crystalline appearance of the platinum salt.

The temperature must be kept low, during extraction and evaporation, avoiding the use of sulphuric acid, otherwise the toxicological examination will show that morphine or one of its derivatives had been taken, and fail entirely to show heroin.

Table III shows the analysis of the organs of dogs poisoned with heroin hydrochloride, giving the amount, in grams, of the alkaloid recovered.

TABLE III.

ORGAN	LAB. NO. 1798	LAB. NO. 1799	LAB. NO. 2077	LAB. NO. 2078
Brain	None	Trace	None	None
Heart	Trace	.0008	.0018	.0023
Lungs			None	None
Blood	.0414	.0257	.0044	.0029
Stomach and contents	.0561	.1011	.3574	.0835
Intestines	.0236	.0481	.0613	.0187
Liver	.0112	.0590	.0042	.0078
Kidneys	.0042	.0075	.0299	.0077
Uterus	Trace			
Urine and bladder			.0411	.0508
Spleen	Trace	Trace	.0005	.0056
Total recovered	.1365	.2422	.5006	.1793

In the above cases where only a trace of alkaloid was found, the tests were characteristic for morphine. The alkaloid extracted from the liver and intestines in all the cases gave a blue with ferric chloride. The blood in No. 2078 did not give the ferric chloride or Prussian blue reactions. In tests where concentrated sulphuric acid is used, as in Husemann's, Froehde's, Marquis', Mecke's reagents, and also in Pellagri's method there is no sharp difference between heroin and morphine. The free base forms white prisms of an alkaline reaction, having a strong bitter taste. The crystals melt at 171° C., beginning to turn brown at about 165° C. In water heroin is nearly insoluble, difficultly soluble in ether and cold alcohol, very easily soluble in hot alcohol, chloroform, and benzol. The free base is precipitated from solutions by ammonia, sodium carbonate, and caustic alkali, soluble in excess of the reagent. A solution of heroin in .5 per cent HCl forms the double platinum salt<sup>3</sup> on the addition of platinic chloride, this is at first amorphous, then assumes the crystalline form like a burr (Figs. 1 and 2) melting point 223° C. with decomposition. The hydrochloride melts at 230° C. Gold chloride gives an amorphous salt when added to a solution of heroin hydrochloride, the salt forming in coalescent globules. According to Danckwortt and Wright,<sup>2</sup> the heating of a water solution of heroin splits off an acetyl group, and there remains monoacetylmorphine.

In the quantitative work all the extracts were repeatedly purified (which entailed a loss at each purification), weighed, and checked with the volumetric method of Elvove,<sup>4</sup> only a lower temperature was used in volatilizing the free acid. Gordin's method gave lower results than the Elvove method. The color-



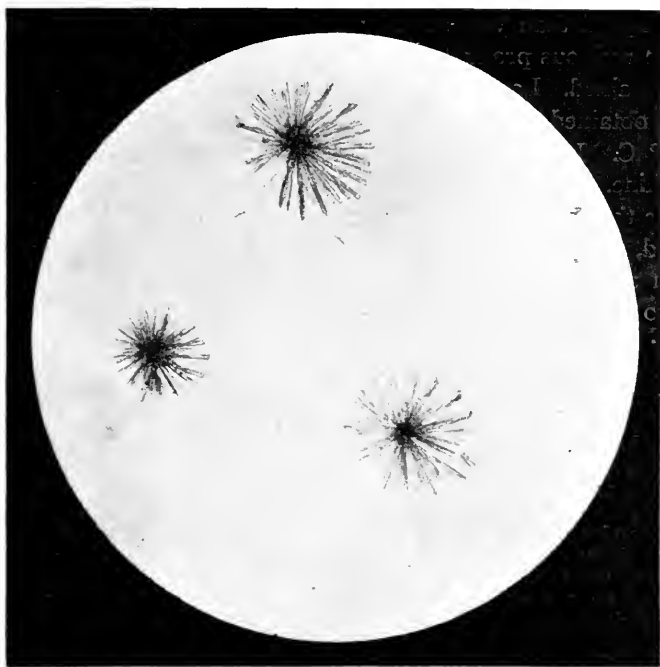


Fig. 1.—Crystals in form of burr, from Heroin Hydrochloride, and Platinic Chloride. Dilutions of 1 to 100.

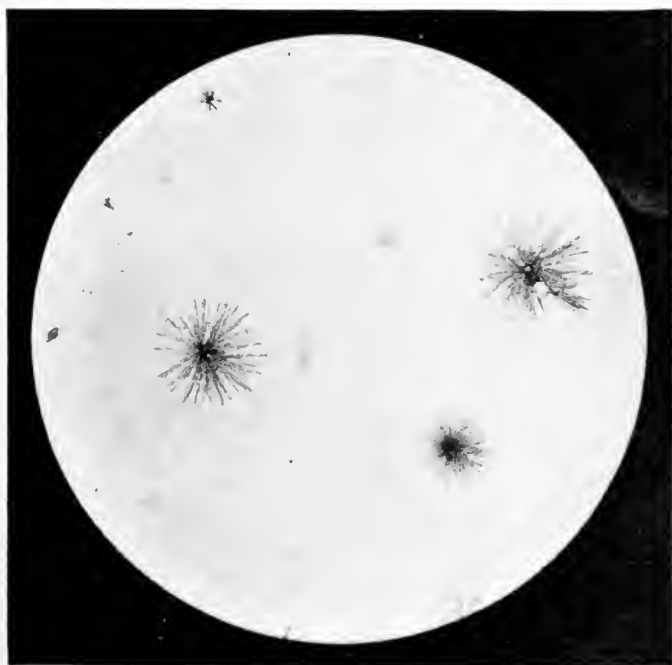


Fig. 2.—Crystals in form of burr, from Platinic Chloride and Heroin Hydrochloride. Dilutions 1 to 100.  
Crystals appearing in two minutes. At greater dilutions a longer time is necessary.

imetric method<sup>5</sup> did not give satisfactory results, as with known solutions more alkaloid was found than was actually weighed out. In most all of the extractions a brown resinous product was obtained, in only one instance was a crystalline residue obtained. Langer<sup>6</sup> extracted the urine of a dog with benzene, filtered the benzene, obtained in the residue after evaporation, heroin with a melting point of 168° C. In the feces of dogs given heroin, intravenously, Langer obtained morphine. The method used undoubtedly caused a deacetylation of the heroin, as the feces was dried, disintegrated, heated with alcohol for twelve hours, filtered, the filtrate evaporated, taken up with hydrochloric acid made alkaline with ammonia, and extracted for 24 hours with benzol.

I wish to express my indebtedness to Prof. W. S. Haines and Dr. John A. Wesener, for helpful suggestions and assistance in preparing this paper.

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<sup>2</sup>Lloyd, John Uri: Personal communication:

"Extract the alkaloid from the suspected tissues by successive extractions with dilute sulphuric acid. Add to the mixed filtrates 'alcresta' (Lloyd's Reagent a form of hydrated aluminum silicate obtained from fuller's earth). Filter the mixture, collecting the alcresta, which is extracted for the alkaloid." This method did not remove the alkaloid completely, the extracts from the tissues carrying too large an amount of extraneous material. This method was used on a sample of urine acidified with tartaric acid, the heroin being recovered unchanged.

<sup>3</sup>Danckwort: Arch. f. Pharm., 1890, cexxviii, 572.

Wright: Jour. Chem. Soc., London, 1880, xxxvii, 610; Ibid., 1874, xxvii, 1031; Ibid., 1875, xxviii, 15, 312, 689; Ibid., 1876, xxix, 652.

<sup>4</sup>Elvove: Hygienic Laboratory Bulletin, No. 54, July, 1909.

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## PREPARATION OF PROTEIN EXTRACTS FOR DIAGNOSTIC CUTANEOUS TESTS\*

BY NEWELL S. FERRY, M.D., DETROIT, MICH.

SO much work is being carried on at the present time from a clinical standpoint, relative to anaphylactic skin reactions with various protein extracts, including the food, plant, pollen and microorganismal, that it has become an important and, in some respects, a serious question as to the most practical form in which to present them. This includes the best method of extracting the crude material, so that all of the available proteins are, according to Goodale<sup>1</sup> "in the form in which they exist when exciting symptoms," the most stable form in which to preserve them, and the most suitable and appropriate state in which to exhibit them for administration.

If the cutaneous method of diagnosing the sensitizations to various proteins is to become of general clinical use, taking its place by the side of the tuberculin, the luetin and other tests, it is evident that the present forms in which the proteins are found should be modified.

In preparing protein extracts for use other than experimental, several things must be taken into consideration. The material, as has previously been mentioned, must be extracted in such a manner as to retain all of the proteins of the foods, plants, and pollen in an available concentrated state; the extract must be preserved from deterioration due to heat, chemical action, and bacterial contamination, and should be so mixed with a nonirritating soluble diluent that it can be used directly by the diagnostician.

There seems to be no dissenting opinion as to the value of water as an extractive, all authorities agreeing that the large majority of proteins are perfectly soluble in water, although it is recognized that such extracts are extremely liable to deterioration. To insure stability of the aqueous extracts, especially toward microorganismal contamination, which appears to be the most destructive influence, various preservatives are used and good results have been reported from all of them. Those most frequently employed are alcohol, glycerin and the phenols.

Goodale advises the use of alcohol in from 12 per cent to 15 per cent dilution, as he believes it best preserves the anaphylactogenic properties of pollen and other protein extracts. He found, however, that the pollen solutions contained one unstable albumen and a relatively stable proteose, the former being readily coagulated by exposure to air. The proteose, on the contrary, suffers but little, and will endure boiling without precipitation. These alcoholic extracts according to Goodale, "should be kept as far as possible from exposure to air, and if in the older extracts cloudiness occurs, it should not be filtered, as thereby the albumen would be removed, leaving only the proteose in solution." It is very evident, if alcohol has such an effect upon protein extracts, that some other preservative would be more acceptable especially when it is desired to use the extracts in an aqueous form.

\*From the Research Laboratory of Parke, Davis & Co., Detroit, Mich.

Several experimenters have obtained satisfactory results with carbolic acid or trikresol and it would seem that these preservatives would be more satisfactory than alcohol, as they not only will prevent bacterial contamination, but, in the small amount in which they are used, have not a deleterious action upon the proteins themselves. Carbolic acid and trikresol, or alcohol for that matter, are not ideal preservatives as the extracts in which they are used can not be applied to sensitive surfaces, such as the conjunctiva, on account of their painfully irritating action. To overcome this, H. C. Hamilton of this laboratory, has suggested an oil of high efficiency which the writer has found best suited for this purpose, as it can be used in a dilution absolutely nonirritating to the most sensitive surfaces and still retain a germicidal action equivalent to either of the other phenols or the alcohol.

As to the methods of preparing extracts for presentation in the most practical form, several suggestions have been offered. The most feasible, up to the present time, depends upon either a direct drying of the aqueous extract or a precipitation of the proteins with alcohol or acetone, followed by a vacuum desiccation of the precipitate. Woodhouse<sup>2</sup> has published a method of preparing desiccated extracts which so far has given favorable results. The aqueous extract is obtained either from cooked (according to method of Goodale) or uncooked foods, and evaporated, if possible, to dryness. The residue is dissolved in sufficient water and precipitated by three or four volumes of 95 per cent alcohol. The precipitate is then centrifuged and washed several times with 95 per cent alcohol, absolute alcohol, and then ether, and finally dried over sulphuric acid. According to Woodhouse, it was necessary to deviate from this general plan by precipitating with a mixture of acetone and ether instead of 95 per cent alcohol. These methods are not entirely satisfactory as the desiccated powder is not always completely soluble in water and the more the dehydration is delayed, the more insoluble becomes the residue.

Baker and Floyd<sup>3</sup> have also proposed a method of desiccating the protein extracts, especially bacterial extracts, which depends upon keeping the extract at a constant temperature of 40° C. with a water bath while rapid evaporation is produced by means of a current of air from an electric fan directly over the suspension. This well-known method of evaporation is always reliable and can be used in any laboratory although the material must be well fortified against bacterial contamination. After the extract has been reduced to a powdered form, it is made into a suspension with glycerin in the proportion of 10 mgs. of the powder to 1 c.c. of glycerin. The authors have this to say concerning their mixture: "One or two drops of this preparation are used for a test. If the powder is applied to the skin directly, a constant standard can not be established or a sterile preparation can not be vouched for. The glycerinated preparation insures, with ordinary care, a sterile preparation and a constant strength, thus establishing a standard."

After trying out several of the most promising of the above methods of preparing extracts, the writer has found no one of them entirely satisfactory, the greatest fault being that they take up too much time to apply; this is quite an essential point when patients are to be tested with a large number of extracts.

The following method has been employed by the writer (the clin-

ical application of these extracts to be published later) during the past year and it has been found most satisfactory as the extracts are better preserved from deterioration and are in a form more convenient to use than any heretofore described. The protein is first extracted from the fresh or dried material with sterile water containing just enough of the previously mentioned highly coefficient oil to act as a preservative. To this aqueous extract is added enough glycerin so that when the solution is evaporated (any one of the several methods of evaporating may be used) the final amount of extract will be in a quantity sufficient to produce a standard previously decided upon. To this glycerin extract is added a sufficient amount of finely ground boric acid powder in a mortar to make a rather heavy paste. For convenience the author has had this paste put up in the ordinary small collapsible tubes, pressing out just the amount necessary for each test.

In applying the paste, the following simple and convenient procedure was suggested and has since been used to the exclusion of all others. Take upon the end of a sterile wooden toothpick just enough of the paste for a test, rubbing the paste into the scarification with the toothpick, which is then to be discarded.

The advantage of this method of preparing the various protein extracts and presenting them for administration lies in the fact that the crude material is first extracted with water, thus assuring the presence of all available proteins; the extract, which can be accurately standardized, is well preserved from deterioration, especially bacterial contamination, and is in a form suitable for instant use. The paste is perfectly miscible with, and soluble in, the body fluids or in water, is not an irritant in itself, and can be used repeatedly either with or without the collapsible tube with practically no chance of contamination.

If it is preferred to obtain the proteins in a desiccated form, according to the method of Woodhouse and others, they can be applied in the paste form by simply making a heavy suspension of the powder in the glycerin before mixing with the boric acid or by mixing the powder directly with the boric acid.

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# LABORATORY METHODS

## STUDIES OF COMPLEMENT: RELATION TO CERTAIN TESTS\*

BY NORMAN E. WILLIAMSON, M.D., STOCKTON, CALIF.

IT seemed necessary to determine the value of human complement for its relation to the author's modification<sup>1</sup> of the Noguchi, and to the Hecht-Weinberg<sup>2</sup>-Gradwohl Test.

The first essential to any accurate study is an exact value for the factors concerned in hemolysis. I would state the present idea of those values as follows:

A measured amount of cells. An amount of amboceptor just capable of hemolyzing these cells in the presence of a measured amount of complement obtained by pooling the serum of two or more guinea pigs. Such a conception lacks scientific accuracy, though for practical purposes it is sufficient for the performance of complement fixation tests.

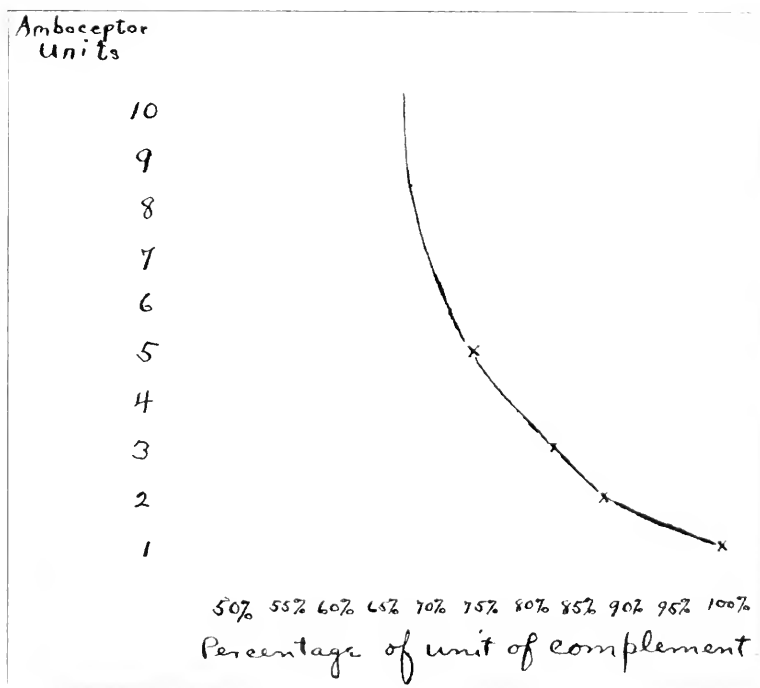
The strength of guinea pig serum has in my experience varied from 0.012 c.c. to 0.03 c.c. as the Noguchi unit. By pooling, it is possible but improbable that an average value would be obtained. All of the sera may be above or below the average. I have had several successive guinea pigs yield serum decidedly above the average in strength. I spent much time in chasing this elusive unit, and have now determined a means of reaching an exact point which can always be replaced.

If one uses increasing doses of amboceptor beginning below the unit in the presence of sufficient complement for the cell suspension, hemolysis will occur steadily in proportion to the amount of amboceptor used. If, however, there is less than a unit of complement for the cell suspension, hemolysis will occur up to a certain point in orderly fashion. Above this point considerable increase in the strength of amboceptor will produce relatively trivial results in increased hemolysis. If, then, one takes several rows of tubes with amboceptor beginning below the unit, steadily increasing in strength from left to right in the same dose in each row and uses complement of different quantities in each row with an interval of complemental volume one-fourth greater than the amount in the next lower row, (provided that one of the rows shall have less complement than the unit), the result will be progressive hemolysis in this row with too little complement up to a certain point, and then relatively insignificant effect of greater quantities of amboceptor. The row above will show complete hemolysis at the point where this change is noted. This will be the exact unit of amboceptor. Exact value for complement is determined by using the intermediate values between these two rows, using 1 per cent variation, the smallest quantity producing complete hemolysis being the unit. The unit relation of factors in hemolysis might then be defined as the least amount of complement

\*From the State Hospital, Stockton, Calif.

and amboceptor for a measured quantity of blood which will proceed steadily in proportion to their strength up to complete hemolysis. Taking such a unit as a basis, I have determined a curve showing the percentage of the unit of complement necessary to produce complete hemolysis in the presence of definite units of amboceptor. It will be seen that in the presence of two units of amboceptor 87.8 per cent of a unit of complement was required for hemolysis; for 3 units, 83 per cent; for 5 units, 75 per cent.

When using high unit values of amboceptor, accuracy can not be obtained though the value can be determined within reasonable limits. Bordet has explained this as deviation of complement due to union of complement to ambo-



ceptor unattached to cells. I consider it just as reasonable and more probable that it is due to the action of the foreign serum (rabbit) containing the amboceptor on the guinea pig complement. Such an action should not occur in human blood between the native antisheep amboceptor and the complement, because both are elements of the same serum. In the presence of large quantities of amboceptor, up to 30 units, no hemolysis in my experience occurred if there were less than one-fourth of a unit of complement.

Complementary values for human serum are determined as follows: First, the hemolytic power of the serum is established similarly to the method used by Gradwohl<sup>12</sup> in his modification, except that 0.2 c.c. of a 1 to 10 serum (0.02 c.c. of serum) and a 1 per cent suspension of sheep cells were used. The relations are the same, but this is much more economical in serum and sheep cells. Next, the units of amboceptor for the amount of cells found to be completely hemolyzed

are determined by using fractions of the serum diluted, with the same amount of cells and sufficient complement (guinea pig serum) for their hemolysis. Thus, for example, one serum in question produced complete hemolysis of 0.8 c.c. of a 1 per cent suspension of sheep cells. The same quantity of cells using sufficient complement were hemolyzed by one-fourth of the amount of serum. This serum contained 4 amboceptor units, sufficient to hemolyze 3.2 c.c. of a 1 per cent suspension of sheep cells.

Now referring to the chart, and following the line of 4 units to the curve of hemolysis and dropping a perpendicular to the base line, it will be found that 18 per cent of complement for 0.8 c.c. of sheep cells must have existed for hemolysis in the presence of 4 units. This would be the unit value for  $0.6\frac{1}{4}$  c.c. practically of a 1 per cent suspension of sheep cells.

Since the amount of serum used is equivalent to the Noguchi unit when using active blood, this can be standardized by reference to a Noguchi unit of sheep cells, that is, 2 c.c. of 1 per cent suspension. Practically the complement present in the serum was 5 12 or about 0.31 of a Noguchi unit. The greatest value I have so far determined in any serum was in one which hemolyzed 0.8 c.c., and contained only two units of amboceptor. Complementary value for this serum would be 0.35 of a unit.

The measure of complement of those sera which are weak in antisheep amboceptor would be determined by adding an equal amount of serum whose values have been determined which is rich in amboceptor. The complemental value of the mixture is then determined and the difference between these and the complemental value of the known serum is the amount of complement in the serum under investigation.

It is evident as before mentioned that in the Gradwohl<sup>3</sup> modification of the Hecht-Weinberg<sup>2</sup> test hemolytic power is all that is measured by Gradwohl. It should never be forgotten that complement is the essential factor which we are measuring in all these tests. More exactly one could determine the amount of complement present in the serum used and use a just proportionate amount of antigen for an accurate test. This becomes somewhat complicated, however. The great value of the Hecht test depends on the fact that one is capable of utilizing a minimum amount of complement in proportion to the syphilitic antibody present. The effect on that complement of foreign sera is eliminated and there is only antigenic action to consider. It is conceivable in the test as they have applied it, however, that the amount of antigen in their dilutions may be less than sufficient to inhibit or absorb all of the human complement present even with a positive blood. Reactions they consider weak because they fail to appear in the high dilutions of antigen may simply lack in antigen, not syphilitic antibody. A useful, simple, qualitative test can be devised by using an amount of antigen which is more than sufficient for inhibition or absorption of the greatest amount of complement present, especially as this amount of antigen in proper dilution has an almost unmeasurable action on any complement.

I mentioned before that I found 0.35 of a unit of complement in a serum. Suppose, until wider experience has shown this to be wrong, that a possible value is assumed for a human complement of one-half the unit in 0.02 c.c. of



serum. The amount of Noguchi antigen (and, by the way, no other except the acetone insoluble fraction should be used with active blood as Noguchi has shown) which is capable of disposing in the presence of syphilitic antibody of two units of guinea pig complement plus whatever human complement may be present is 0.1 c.c. of a 1 to 10 dilution. The largest amount of antigen it would then be necessary to use would be  $5/25$  of  $1/5$  of this amount for 0.02 c.c. of serum. To overcome any physical effects of concentration it is desirable to use 0.5 c.c. of a 1 to 250 dilution. I have found, because of its sure content of anti-sheep amboceptor, 0.03 c.c. of serum to be a preferable amount to be used, and for this I use 0.75 c.c. of a 1 to 250 dilution. The serum is diluted 1 to 10 as before; 0.3 c.c. placed in front and back tubes, 0.75 c.c. of 1 to 250 antigen in the front tube, and 0.75 c.c. salt solution in the back tube, and incubated one-half hour. Then add 0.15 c.c. of 1 per cent suspension of sheep cells and incubate one-half hour. It is exceedingly rare to find any sera so weak in anti-sheep amboceptor that 0.03 will not hemolyze 0.15 c.c. of a 1 per cent suspension of sheep cells, which serve only as an index of absolute absence or presence of complement. Any quality, however small, that will show this constantly, is the right quality to use. Negatives by this test are of the highest value. Positives give little information of the amount of syphilitic antibody present, and may be erroneous. For positive bloods the simplest and best test which will show any number of units is my modification of the Noguchi test<sup>4</sup> and its titration sequel.<sup>5</sup>

Experience proves that human complement could be disregarded in this test as was stated by Noguchi,<sup>6</sup> though an effort was made to measure it in tube 4 with antihuman amboceptor. The fallacy of measuring human complement with cells of the same genus led to this investigation. I was anxious to determine exactly how much complement was being disregarded. I have now performed nearly a thousand tests checked by history, clinical findings, and, wherever indicated, by spinal fluid study, and find that the portions advised are satisfactory. I can not corroborate the statement of Busila<sup>7</sup> that no positive reaction has been obtained in the cerebral spinal fluid in a case which was negative when using active blood. I have had a case which was negative by my modification of Hecht; also by Hecht-Gradwohl and by the modification of Noguchi which gave complete inhibition using 0.2 c.c. of cerebral spinal fluid. It is necessary to study the spinal fluid in every positive or suspicious case.

Referring again to the curve, it is interesting to know its bearing on the Wassermann reaction. In the Wassermann reaction 2 units of amboceptor are used. The serum referred to above, containing 4 units for 0.8 c.c. of 1 per cent suspension of sheep cells, would have contained in the amount used in the Wassermann 3.2 units of anti-sheep amboceptor. There would then have been 5.2 units of anti-sheep amboceptor in the test. Two units require 87.8 per cent of complement for complete hemolysis. Five units require 75 per cent. The error due to excess of amboceptor in such a case is trivial. The principal objection to the Wassermann must be considered, as repeatedly emphasized, the destruction of syphilitic antibody by heating the serum. The influence of the heat on complement during the preliminary incubation in complement fixation reactions is of considerable interest. I have found that where the complement is incu-

bated with the cell suspension as in the Noguchi test for one-half hour at  $37^{\circ}$  C. from 0.4 to 0.5 of its strength is lost. This is a factor of serious moment in the titration of antigens. Not 2 units of complement, but the quantity of complement which after heating would still have a value of 1 unit, should be used in antigenic titrations. Using 2 units of amboceptor in the antigenic titration 87.8 per cent of a unit of complement would be necessary for complete hemolysis as has been shown. Probably about 15 per cent of the complement is lost as a result of the action by the antigen in the dose which proves slightly anticomplementary. The correct dose of antigen would have an anticomplementary action of from  $\frac{1}{4}$  to  $\frac{1}{3}$  of this.

#### SUMMARY.

The standard unit of hemolysis is the least amount of complement and amboceptor which will completely hemolyze a unit suspension of blood: it can be determined by cross titration as described.

Based on this unit, a curve showing percentage of complement required for multiple units of amboceptor is presented.

By reference to this curve human complement may be measured. Hemolytic power of the serum for sheep cells is determined; amboceptor units for the same amount of cell suspension; reference to the curve gives complemental value.

Antigen just corresponding to the complement can be used in the Hecht-Weinberg-Gradwohl test instead of using hemolytic power alone as described by Gradwohl.

A more useful, simple test can be made by a modification of the Hecht, as shown, for the great value of the negatives.

For bloods containing more syphilitic antibody the author's modification of the Noguchi and its titration sequel are the best and simplest tests.

The error of the Wassermann due to excess of antisheep amboceptor is not great; inactivation is the most serious objection.

Antigens should be titrated with just sufficient complement to allow 1 active unit after heating for one-half hour at  $37^{\circ}$  C.

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## EDITORIALS

### *The Desirability of Having Expert Entomologists in Military Camps*

INSECT agencies in the spread of disease are now fully recognized. Typhus fever, yellow fever, malaria and bubonic plague are transmitted only or chiefly by the bites of insects. The specific organism of typhoid fever, dysentery, tuberculosis, and other less prevalent diseases may be transmitted by insects. In some sections of this country, notably in the Southwest, there are certain noxious insects. Apart from the transmission of disease, insects are annoyances, cause loss of sleep, and may add much to the discomfort of the soldier and greatly impair his efficiency. Moreover, insects may transmit glanders and probably other diseases among horses and certainly lead to much discomfort and loss of rest among these animals, so essential to our army. Still further, insects sometimes destroy large quantities of military stores, especially foods.

The importance of the house fly in the dissemination of typhoid fever in the military camps in 1898 was pointed out and emphasized by the special Board appointed to investigate the causes of the wide prevalence of that disease. The following is quoted from the report of the Board:

"We are satisfied that the evidence furnished in our studies, to be detailed

later, is sufficient to show beyond reasonable doubt that the most active agents in the spread of typhoid fever in many of the encampments in 1898 were flies. The reason for coming to this conclusion will be given in detail later, but may be summed up here as follows:

1. The latrines contained fecal matter specifically infected with typhoid bacilli.

2. Flies alternately visited and fed upon this infected fecal matter and the food in the mess tents. More than once it happened, when lime had been scattered over the fecal matter in the pits, that flies with their feet covered with lime were seen walking over the food.

3. Typhoid fever was much less frequent among members of messes who had their tents screened than it was among those who took no such precaution.

4. Typhoid fever gradually died out in the fall of 1898 in the encampments at Knoxville and Meade with the disappearance of the fly, and this occurred at a time of the year when in civil practice typhoid fever is generally on the increase."

The fly problem has not yet been satisfactorily disposed of. In some of the camps along the Texas border in the summer and fall of 1916 these pests were abundant, especially was this true about El Paso. Flies swarmed over and on the camps and not only annoyed the men, but according to the medical officers, a severe and widespread epidemic of amebic dysentery was undoubtedly spread by these insects. In part at least this was due to the filthy condition of the civilian population adjacent to these camps. Over these, the medical officers had no control, and local health officials were inefficient or indifferent or both. The breeding of flies in the regions about these camps rendered their exclusion impossible. This was not the only ill supplied by the surrounding civilian territory, in which red light districts and other menaces to the soldier flourished abundantly. From this we should take a lesson in establishing camps for the present war. About each camp there should be a zone, large enough to be effective, under national patrol and control. It may be that the policing of such zones should be a function of the Public Health Service. It is certain that the sanitation of such areas can not be safely left to local, civilian control.

Medical officers on the Texas border report that with flies abundant as they were last summer in some of the camps they were unable to construct fly-proof latrines. Evidently the only thing to do is to prevent the breeding of flies not only within the military lines but in the adjacent country. This is feasible, but is not likely to be done unless the sanitation of the surrounding country be placed under federal supervision. This will result in the protection of not only the military forces but of the civilian population. The army reduced the death rate in Havana below that of any city in this country and that of the canal zone below that of any State in the Union. If the sanitation of the whole country could be administered for a few years by federal authority the result would be beneficial.

The bite of the stable fly is more vicious than that of the house variety and may draw blood. It breeds in moist hay and straw and is a danger not only on account of the poison caused by its bite, which often drives horses and mules

frantic, but on account of its ability to spread disease among these animals. The charge that this fly spreads poliomyelitis is lacking in proof, but it may spread glanders and other diseases among horses. Since it does not, often at least, develop in manure, the procedures necessary to prevent its breeding are different from those efficient with the house fly.

Bedbugs sometimes render the life of the soldier at night almost unbearable and when a camp becomes badly infected the eradication of this pest is a difficult task. It has been found necessary to immerse cots bodily in a boiling dilute solution of caustic soda. So far as known this insect is not specifically responsible for the transmission of any diseases prevalent in this country, but this is a world war and there is no telling to what lands our soldiers may be called. Besides, the annoyances and irritation caused by these insects should be avoided. The cone-nose bedbug is found in Mexico. It is a blood-sucking insect, and sometimes is an inch long. It flies at night and occasionally bites man, causing much irritation and swelling and necessitating antiseptic treatment.

Fleas breed in dirt and many of the Mexican adobe houses are badly infested. Whether these fleas may spread bubonic plague or not is not definitely known.

The chigoe, not to be confused with the chigger, or bedbug, is a kind of flea. The females penetrate the skin, usually under the toe nails or on the soles of the feet, and cause marked swelling. The insect should be removed surgically and aseptically.

With the exception of the Rocky Mountain ticks, which may spread the spotted fever observed in certain localities in the West, we know of no disease in man transmitted by this insect in this country.

Centipedes carry poison glands at the base of the front pair of legs. The bite may be followed by distressing symptoms.

A poisonous spider, usually known as a tarantula, is frequent in warm countries and especially in Texas and Mexico. There is also a little black spider, with a red spot on its abdomen, whose bite is poisonous.

Some of the best camps in Texas were not free from ants, and the so-called "harvest" variety attacks man and the sting is exceedingly painful.

The soldiers in Cuba in 1898 were annoyed by a small scorpion found among the rocks. The sting is painful but not serious. This little beast seems to have a special fondness for leather and is attracted by shoes, harness, etc.

Cockroaches often come in numberless legions and may destroy much food. They seem to be wholly immune to formaldehyde, but are quickly destroyed with commercial sodium fluorid, either pure or diluted with an equal portion of some inert substance, as flour or gypsum.

Certain fly larvæ may develop in the living body, and this condition is known as myiasis, which may be intestinal or dermal. The most common insect finding its way into, and growing in, the intestines of man is the maggot of the cheese fly, usually called the cheese skipper. However, the maggots of the blow fly and the common house fly have been found in the intestines of man.

Dermal myiasis is more common and may occur in a diseased nose, or in

neglected wounds. The so-called screw-worm fly usually breeds in dead animals, but it may establish itself in wounds and has been known to cause death.

One of the most brilliant, recent discoveries is that of the French Army Surgeon, Nicolle, who demonstrated that typhus fever is transmitted from man to man by the bites of lice. Man serves as host to three species of lice. *Pediculus capitis* and *p. pubis* seem to have preempted certain portions of man's body as sources of their food supply and for the location of their homes and the breeding of their kind. The third is known as *p. corporis* or *p. vestimenti*. The former name indicates its cosmopolitan character and the latter shows that its chief abiding place is in man's clothing. This is the cause which transmits typhus fever. It was well known among the soldiers of the civil war by the appellation of "gray-back," but at that time it carried no typhus virus. This species was also abundant on the Texas border in 1916, but again was without specific infection. Soldiers should not be permitted to harbor lice of any species, with or without typhus infection. *P. capitis* deposits its eggs or nits on the hair some distance from the scalp and clipping the hair removes most of them. The remainder are easily destroyed by a shampoo with 2 per cent carbolic acid. One thorough inunction of its special region, with blue ointment usually destroys the pubic residents. *P. vestimenti* is destroyed with more difficulty and is a more dangerous guest. English and French physicians have given much attention to this parasite during the present war. Shiply has gone painstakingly into its life history, discovering its habits in feeding and breeding, etc. Others have given chief attention to means for its destruction. From what has been said it must be evident that the eradication of these pests can not depend wholly upon cleanliness of body, but the verminicides must be applied to the clothing as well. Indeed it often happens that not a louse can be found on the stripped body while the clothing may be swarming with them. Furthermore it must be evident that a verminicide suitable for application to the body may be ineffective when applied to the clothing, and since every article of clothing from head to foot, and bedding as well, may be infested, the solution of the verminicide and the method of application must vary with the fabric. In order to be effective the nits as well as the lice must be destroyed.

It is not our intention to review all the agents employed in the destruction of lice. Clothes not injured by this process, may be steamed for twenty minutes under pressure. A benzine bath, as employed in dry cleaning, is said to be effective. A soap containing 35 per cent cresol and 65 per cent naphtha, and another containing 35 per cent xylol and 65 per cent naphtha have been recommended by a French authority.

Yellow fever, malaria, filariasis and dengue are disseminated by mosquitoes. The different scientific departments of the Government have issued from time to time most excellent monographs on these pests, one of which written by Ludlow is entitled "Disease-Bearing Mosquitoes of North and Central America, the West Indies and the Philippine Islands." This is most thorough up to the time of writing (1913) and we understand that a new edition is

to be issued soon. The author states that in the prevention of diseases spread by these insects two problems must be solved.

1. What disease-bearing mosquitoes are present? This divides itself into: (A) What anophelines are present; (B) Are *stegomyia fasciata* *eulex fatigans* or *Mansonia uniformis* and *africana* present? 2. How shall the species found be exterminated? It will be seen that both of these questions demand the services of an expert. Only an expert knows enough to find and identify the different species and to exterminate the species found. They should be found early and before they become numerous. Having found the breeding places, how shall the propagation of these insects be prevented? This depends upon the species and the locality. "Each land, each locality, each species presents its own problem, and until more is definitely known of the habits of the individual species, each demands a solution from the worker in his own field."

We have gone thus fully into these matters in order to show the desirability of employing the services of expert entomologists in our military camps and with our armies on the move. There is probably no country in the world better supplied with experts in this line. The Surgeon General of the Army has already rendered his country most distinguished service and made himself the greatest authority in the world in the suppression of yellow fever and malaria. There are others in the army medical corps as Craig, Darling, Russell, and Nichols who have been investigators in this field. In the Agricultural Department we have Howard and those whom he has trained, some of whom are now serving as state entomologists or as professors in universities.

The Russian army has suffered great economic loss in the destruction of its food supplies by weevils and has called upon its entomologists to prevent further loss.

The English, French, Russians, and Germans have established commissions of entomologists. We should have their expert knowledge to start with. For many years this country has recognized the importance of protecting crops from the ravages of insects. Let us have the protection and help of these specialists for our soldiers.

The following points seem clear:

1. Insect-carriers of diseases have wrought havoc in armies.
2. The early recognition of harmful insects require expert knowledge.
3. Their eradication promptly and efficiently demands the same experts.
4. The knowledge gained by a corps of skilled entomologists would make a valuable contribution to science, and be of value in the future in both civil and military life.

L. C. F.

*Suggestions and Recommendations Concerning the Physical Examination of Recruits for the United States Army and Navy, and for the Collection of Scientific Data and Material in Connection With the Hospital Service*

THE eminent anthropologists of the Smithsonian Institution, Holmes and Hrdlicka, have submitted to the National Research Council some suggestions under the above heading. They say: "Soon a multitude of young men will be called in the organization of the new army and navy of the United States, and since good health with physical fitness are the foremost requirements of the soldier and sailor, the men called should be subjected to most careful examination. The object should be the selection not of the tallest and strongest men, but of the thousands who, on the basis of our best physical and physiological knowledge, may reasonably be expected to be fit for the tasks to be imposed upon them, or who may be made fit during the period of training. Certain standards relating to the physical development and to the health of recruits have long been established and are now employed in our recruiting offices. These differ somewhat in the different branches of service. They are all right in normal times, but they take no account of racial differences in physical characteristics and give little consideration to the possibility of improvement in the individual that should follow six or nine months of training, medical supervision, and outdoor life."

Going over the regulations for the army of the United States, these men recommend that the following "he can not speak, read and write the English language" be changed into "he does not possess a fair speaking knowledge of the English language." In other words, a man should not be excluded from the army because he can not correctly and fluently speak the English language. It is only necessary that he should be able to understand and intelligently grasp plain instructions and commands issued in this language. The present regulations say that the recruit is expected to have twenty sound teeth including four opposite incisors and four opposite molars. As the prevalent defective condition of the teeth in many cases is connected with the food and other habits of modern civilization, or to accident, rather than with disease, and as in a large majority of cases the condition of the teeth can readily be corrected by dentists, it would seem best that this stipulation be replaced by one permitting of more latitude in this direction. Possibly the following would meet the requirements: "The recruit is expected to have twenty sound teeth, including four opposite incisors and four opposite molars, but if his physical condition is otherwise satisfactory, he may be accepted even though below this dental standard, provided the defects of the teeth are such as can be readily corrected by army or navy dentists."

In regard to the stature requirements, it may be said that the present minimum in every branch of the army or navy is five feet four inches, and in the case of mountain artillery, this minimum is five feet eight inches. The minimum for the English infantry and some other branches of the service, prior to the present war, was five feet two inches, but it has since been reduced. On the Continent the minimum differs according to place. There are a number of European nationalities in which the average height of the adult male reaches but a few



tenths of an inch over the minimum of the United States. These nationalities, most of which are well represented in this country, include the Italians, Greeks, French, Swiss, many of the Slavs, Magyars, Russian and Austrian Jews, Rumanians, Lithuanians and Germans. Should the present minimum in stature for the United States army and navy be adhered to, from two-fifths to more than one-half of the men belonging to, or descendants from, the nationalities mentioned above would be excluded. In view of these facts and since short stature in a large majority of cases signifies normal variation rather than degeneration, it appears advisable that the minimum be reduced for all branches of the service to sixty inches, and that corresponding to this the minimum weight requirement be reduced from 128 to 120 pounds.

Holmes and Hrdlicka call attention to the desirability of collecting material for anthropological research during the present war. Efforts in this direction were made in the northern army of the United States during the Civil War and the results of these studies are embodied in three volumes of valuable data by Baxter and Gould, and in certain collections now in the Army Medical Museum. Our army will include not only people of many nationalities but also those of different races such as Indian, Negro, Filipino, and possibly Japanese and Chinese. By a study of both the living and the dead, information of great value may be obtained and our knowledge of anthropology may be greatly extended.

—T. C. F.

### *A New Mercurial Germicide*

SCHAMBERG, Raiziss, and Kolmer, of Philadelphia, have presented to the National Research Council results which they have obtained in the preparation of a new mercurial germicide. This has been formed by the introduction of mercury into the phenol group and the trade name proposed for it is "Mercurophen." The above mentioned investigators have made a thorough study of this new compound, and have stated the results of their investigation in the following conclusions:

1. Mercurial compound No. 99 (mercurophen) exhibits against the staphylococcus aureus, in the "antiseptic test," fifty times greater activity than mercuric chloride; it destroys these bacteria on prolonged exposure in bouillon in a dilution of 1:10,000,000.

2. In a menstruum of ascitic fluid, mercurophen is two hundred times more germicidal against the staphylococcus aureus than mercuric chloride.

3. By the Rideal-Walker method, mercurophen exhibits ten thousand times greater germicidal power against the *Bacillus typhosus* than phenol, and over thirty times greater activity than mercuric chloride.

4. Mercurophen disinfects the hands in dilutions of from 1:10,000 to 1:40,000 in one minute, whereas mercuric chloride in a dilution of 1:50,000 requires over five minutes, and in a dilution of 1:10,000 requires over fifteen minutes.

5. Mercurophen sterilizes ordinary rubber tubing in thirty minutes in a dilution of 1:100,000. Mercuric chloride accomplishes this result in a dilution

of 1:16,000. With tubing heavily infected with cocci and spores, mercurophen required a 1:500 solution and mercuric chloride fails in a 1:50 solution.

6. Mercurophen in a 1:5000 solution sterilizes feces in thirty minutes; mercuric chloride accomplishes this result in a 1:2000 solution.

7. The precipitating effect of mercuric chloride on human serum proteins is four to five times greater than that exhibited by mercurophen. This is an obvious advantage possessed by the latter substance.

8. Solutions of 1:5000 of mercurophen exhibit no evidence of tarnishing on nickel-plated instruments after twenty-four hours exposure.

9. In experiments not detailed here, mercurophen administered intravenously in rabbits has a lower toxicity than mercuric chloride.

—I. C. F.

### *Digitalis Grown in America*

SOME months ago the Chairman of the Committee on Medicine and Hygiene of the National Research Council took up the question of digitalis and belladonna grown in the United States. It is generally understood that we are dependent upon importation of these very valuable drugs from Europe. In the present state of medical science it would seem quite impossible in the treatment of certain diseases to get along without preparations of digitalis. It is very gratifying, therefore, to ascertain that digitalis has been grown experimentally at the Universities of Minnesota, Wisconsin, and Oregon. Wulling, Dean of the Pharmacy School of the University of Minnesota, states that he now has about one hundred quarter-pound jars of digitalis purpurea of the 1916 crop. Furthermore, he adds that he would be glad to place at the disposal of the government fifteen or twenty pounds of this digitalis in coarsely powdered form. He states that this would exhaust his supply except what the hospital and dispensary of his own university would need. Concerning this year's crop he says: "We probably could arrange to increase our planting to a point to enable us to furnish at the end of the season a somewhat larger supply than we have on hand now. The powdered digitalis we have on hand would be suitable for percolating, but not for prescription work. For the latter, we could reduce the powder to a much finer state of subdivision by remilling it."

Rowntree has already published some articles on the physiological action of the digitalis grown in Minnesota. He shows that this is quite equal to, if not better therapeutically than that grown in other countries. Morris has experimented still further with the Minnesota grown digitalis.

There are many varieties of digitalis. Heretofore the variety most used is digitalis purpurea, but Morris thinks that digitalis lutea is quite as efficient in its action on the circulatory organs and is much less irritating. It seems, therefore, that the Minnesota grown digitalis may in the near future wholly supplant the imported article. The necessities growing out of the present war will probably lead to many similar discoveries, and after a few years this country will be more independent than it has been in the growth of medicinal plants and the preparation of therapeutic agents from them.

—I. C. F.

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## ORIGINAL ARTICLES

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### THE BACTERIOLOGY OF POLIOMYELITIS

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#### LABORATORY CULTURE MEDIA AND METHODS.

IN all bacteriological investigations it is essential to have standard methods which have been thoroughly tried and which are sufficiently numerous and different to enable one to adduce and detect variations in the behavior of any organisms which we may encounter. Although within the forty years which, approximately, cover the history of bacteriological discovery many methods for the laboratory cultivation of bacteria have been devised, yet there are but few which represent such really distinctive conditions as serve to distinguish one organism from another or which serve to cultivate an organism while others fail. The failure of the usual laboratory procedures to grow particular recognized parasites has been accounted for by the statement that living tissue or the conditions of the animal body were essential for its well being. However, the introduction of the fresh tissue media, as in the cultivation of the spirocheta pallida, removed the difficulties in the case of several organisms, and led bacteriology a step further ahead. In the employment of fresh tissue, such as the kidney of the rabbit, so long as we are dealing with an organism which is in no danger of being confused with contaminants one is entirely safe, especially when but one culture medium and method is followed and experiments are carefully controlled. But should we be searching for an unrecognized or a but partially known parasite, and in the course of our study transfer cultures from one form of medium to another, as from anaerobic serum plus fresh tissue, to aerobic solidified serum, we will meet unexpected difficulties since organisms introduced in the piece of fresh tissue that, perhaps, represent the flotsam-and-jetsam that fall into an animal's blood stream, frequently develop in one or the other of our

culture media and cause much trouble. Thus I have commonly found a contaminant of rabbits' kidneys when they are crushed and smeared over Loeffler's blood serum and the medium incubated aerobically—especially when treated as in the following.

A NEW METHOD.—These observations caused me several years ago to attempt to devise a substitute for the fresh tissue, and in so doing it was reasoned that since one known difference between living blood plasma and blood serum was the separation of the fibrin, and that since in this process a precipitation in which a calcium compound was involved was understood to take place it might be well to try the usefulness of blood serum (sterilized by the fractional method) plus a soluble calcium salt. Reasoning that as in the animal economy the cir-

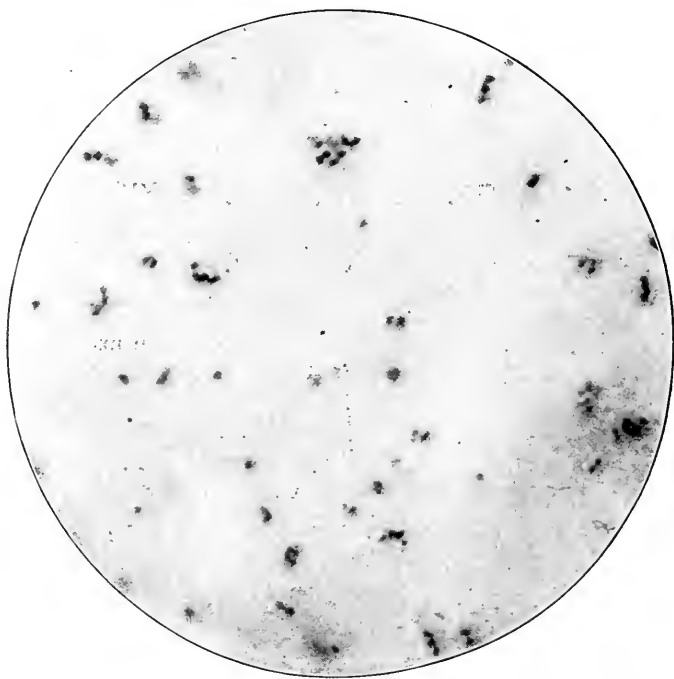


Fig. 1.—Smear from culture made from cord of Case I. Grown 4 days, anaerobically, in 25 per cent hydrocele fluid mixed with 75 per cent bouillon-limewater (equal parts of each). Methylene blue stain. Magnification, 1500. Photograph reduced one-fourth.

culution removed all acids, including carbon dioxide, it was thought that calcium oxide was the best available lime salt, since it was sufficiently soluble to supply a calcium base and would also fix any acids developing, especially the carbon dioxide. Various experiments with this medium (blood serum and lime-water) developed the fact that a diluent of bouillon, in various proportions, greatly enhanced its suitability for various bacteria. Modifying this combination to the cultivation of bacteria upon a solid surface (as Loeffler's blood serum) it was found that to the desirable properties of both media could be added that of a continuously wet surface, by depositing a few drops of the serum-bouillon-limewater at the bottom of the slanted Loeffler serum tube, just prior to its inoculation, and tilting the tube once or twice a day, while in use, to cause the fluid

to flow over the inoculated surface, a procedure which also might be thought to act similarly to the blood current in mechanically washing away bacterial waste products. This method was employed extensively in the experiments to be detailed.

**SOURCE OF CULTURE.**—Cord and brain specimens from autopsies on eight fatal cases of the recent New York City epidemic were used. In five instances the specimens were from one to six weeks old and had been preserved in 50 per cent glycerine and kept on ice. In three instances specimens of brain and cord tissue direct from autopsies were employed. Specimens from one of the cases showed contaminating organisms and, as a precautionary measure, the cultures were discarded.

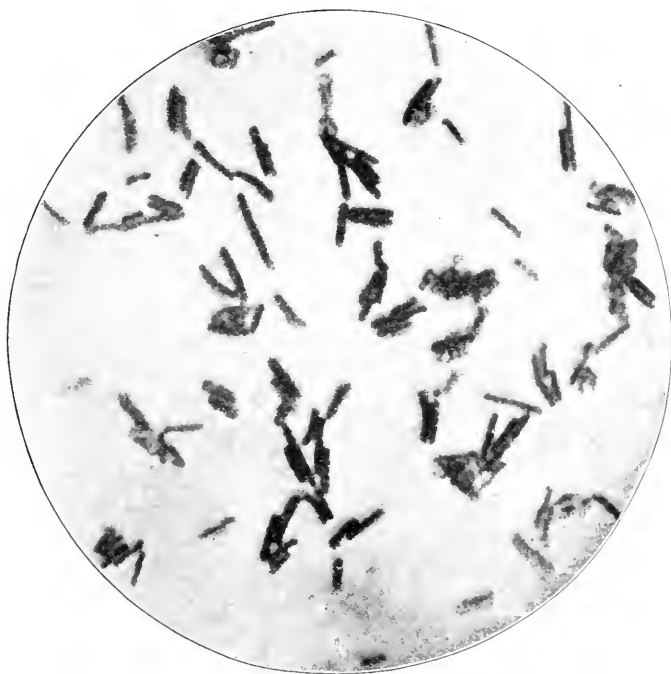


Fig. 2.—Smear from culture made from brain of Case IV. Sixth generation, grown two days, aerobically, on Loeffler's blood serum, washed with bouillon-limewater (lactose 4 per cent). Methylene blue stain. Magnification, 1500. Photograph reduced one-fourth.

Later, for the purposes of comparison, a culture isolated by a western bacteriologist was used.

**METHODS OF CULTIVATION.**—Series of cultures were made from all specimens: (A) In the Flexner-Noguchi fresh tissue medium; (B) in human serum (hydrocele fluid) 25 parts, diluted with 75 parts of 50 per cent limewater in nutrient bouillon,\* and (C) on Loeffler serum tubes wet, as described, with the hydrocele-bouillon-limewater. (D) Control cultures were made on plain nutrient agar, and on plain Loeffler's serum (solidified).

\*Lactose, 2 per cent, was also added, but later proved nonessential. The proportion of hydrocele fluid was also increased in some cultures.

Incubation was made at 37.5° C., anaerobically (media covered with liquid paraffine) in (A) and aerobically in (B), (C) and (D).

CHARACTER OF CULTURES OBTAINED.—Cultures from both brains and cords of the seven cases considered (made by dropping small pieces of each in fluid cultures, and by smearing pieces over the surfaces of the solid media) and the western culture—in all, fifteen cultures—gave results as follows:

Flexner-Noguchi medium (A): In 100% hydrocele fluid, plus a piece of fresh kidney, a slight cloudiness of the media developed by the third day, and smears made at this time showed a pleomorphic organism identical in appearance with that in the microphotographs illustrating Noguchi's article in the *Journal of Experimental Medicine*, October 1, 1913. Appearances were similar in smears



Fig. 3.—Smear from second generation of culture shown in Fig. 2. Planted on Loeffler's blood serum which, at the end of two days—no growth having appeared—was submerged in bouillon-limewater (dextrose 1 per cent). Smear made one day later. Gram's stain. Magnification, 1500. Photograph reduced one-fourth.

from all of the fifteen cultures. Limed Serum Bouillon (B): Cultures made in this medium grew much better than in the foregoing and showed a distinct cloudiness on the second day. Smears gave an organism similar to that before mentioned. Loeffler Washed with Limed Serum Bouillon (C): By the end of 48 hours, small, pinhead sized colorless colonies appeared on the surface of the medium. Smears made at this time showed an organism very similar to that produced by the other methods. When, however, the washing and incubation was continued another 48 hours, slight digestion (peptonization) of the medium began, and smears made at this time showed a sporulating bacillus, that is one that stained bipolarly, and of which the polar bodies were to be seen, some still within the bacilli, some in the act of escaping from the poles of the bacilli, and some en-

tirely free from the parent form.\* Occasionally clubbed-shaped bacilli, similar in appearance to sporulating tetanus bacilli were encountered (Fig. 3). Later than the fourth day all bacilli had involuted, showing as hollow ovoids, such as would usually be denominated spores. Yet since the polar bodies, which are evidently the germinating elements, tend to escape at this time, this name in its accepted meaning is hardly applicable. Nutrient Agar (d): At the end of a week no growth had appeared. (The same was true of cultures made upon plain Loeffler blood serum).

**TRANSPLANTS.**—While the first generation of all cultures, either in the Flexner-Noguchi medium, in the limed-bouillon serum, or on the Loeffler, washed with the same, gave organisms the only difference between which appeared to be a variation in size, and which were Gram-positive, transplants seemed to indicate actual biological variations. Six cultures (numbered 1 to 6) from as many sources were conducted through a number of generations, on various media, with results as follows:

*Culture 1.*—Replanted from washed Loeffler to plain solidified Loeffler gave, within 24 hours, a creamy growth which by the end of the third day became slightly wrinkled and showed slight liquefaction of the medium. The organism appeared as small Gram-negative bacilli, of which some showed one or two Gram-positive poles. By the third day the bacilli with polar bodies "sporulated,"—assumed an oval nonstaining form, from which the polar bodies were often seen escaping. By replants, made at the end of 24 hours' growth, this culture was continued for eleven generations of identical qualities. The eleventh generation was then transplanted to nutrient agar, and within 24 hours developed as a white scum containing organisms which varied from both large Gram-negative and Gram-positive bacilli to diplococci of similar staining variations.

A replant of the last mentioned culture to bouillon-hydrocele-limewater gave a heavy growth of similar organisms. A replant from the fluid culture to Loeffler's solidified serum gave a distinctly wrinkled scum of mostly Gram-positive bacilli which proceeded to "sporulation" and marked liquefaction of the medium by the third day.

*Culture 2.*—Replanted as with Culture 1, gave at the end of 24 hours a wrinkled white scum of mostly Gram-positive bacilli, which "sporulated" and liquefied the medium by the third day. The same results were continuously produced for eight generations. Replant from the sixth generation into bouillon-hydrocele-limewater gave, by the end of five days, a faintly cloudy medium containing a good many minute Gram-positive diplococci and streptococci.

*Culture 3.*—Replanted, as above, for eleven generations, gave at the end of 24 hours a creamy, but partly wrinkled, scum containing large Gram-negative bacilli, among which could be found a good many Gram-positive ones. Liquefaction of medium varied from none to marked, by the third day. A replant of the eleventh generation into bouillon-hydrocele-limewater gave, by the fifth day, a few Gram-positive diplococci.

*Culture 4.*—Replanted as above for eight generations gave, at the end of 24

\*Since writing this there has appeared (Jour. Bacteriol., March, 1917) a very interesting article by Mellon, describing the metamorphosis of a diphtheroid bacillus into streptococcus-like formations.

hours, a creamy growth of large bacilli most of which were entirely Gram-negative. A few, however, were always Gram-positive or had Gram-positive poles. In most cases there was slight liquefaction of the medium by the second day. The eighth generation, replanted into the fluid medium last mentioned, gave, by the fifth day, a few Gram-negative diplococci. A replant of this culture left at room temperature for several days developed two lemon yellow colonies of large Gram-negative diplococci—indicating a very interesting and perhaps most important biological variation.

*Culture 5.*—Replanted as with the foregoing, gave a slight creamy growth, which, for ten generations, failed to show the slightest liquefaction of the medium, and which consisted almost entirely of large Gram-negative bacilli (an occasional Gram-positive pole could usually be found). The tenth generation, replanted into the fluid medium previously mentioned, gave a growth containing organisms

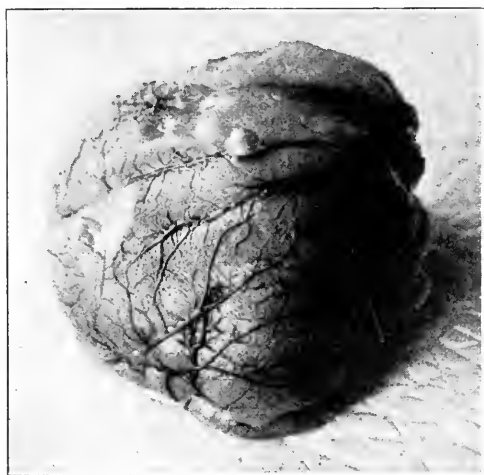


Fig. 4.—Cerebrum of young guinea pig which contracted a polio-myelitis bacillus infection from contact with another guinea pig intraperitoneally inoculated with the organisms of Culture 4 taken from the throat of an experimenter accidentally infected with this culture. One platinum loop of throat mucus in 0.5 c.c. saline was used.

varying from Gram-negative bacilli to Gram-positive diplococci. By replanting the eighth generation into bouillon and then returning it to Loeffler, a creamy and slightly wrinkled growth was obtained, which produced a slight liquefaction by the second day.

*Culture 6.*—Replanted from "blood agar" (on which it had been grown as Gram-positive streptococci) to Loeffler, washed as detailed, gave, by the fourth day, many small white colonies containing coccoid bacilli which were intermediate in the way in which they were affected by Gram—they were neither distinctly positive nor negative. A replant to the same medium gave, by the fourth day, a growth which began to show liquefaction and in which the organisms of the softening areas had developed to the "sporulating" large Gram-positive bacilli. From this last culture the same form (large Gram-positive sporulating bacillus) was propagated on plain Loeffler for ten generations and then transferred to serum (plural exudate) to which a small amount of calcium oxide had been added.



Cultivated in this for six generations (five days a generation) a growth consisting almost entirely of fine Gram-positive diplococci was developed.

GENERAL CONSIDERATIONS OF VARIATIONS OBSERVED.—*Form*.—It is seen from the above that growth in serum-containing fluid media tends to cause the organism to assume the coccoid form, even after extensive cultivation as a distinct bacillus on other media.

Development to the sporulating, Loeffler liquefying, bacillus is seen to be easily secured by growth upon this medium washed with limed-hydrocele-bouillon mixture.

It should be emphasized that for the complete transformation of the coccus form into the bacillus form of the organism, or vice versa, sometimes many generations are necessary. The sporulating bipolar bacillus evidently is the fully developed organism, and the tendency of all cultures is to develop into this. Therefore, it is easy to derive the bacillus from the coccus form, but more difficult to change a culture in the bacillus form into the coccus form. Thus a re-plant of the bacillus form in serum (fluid) often grows with difficulty, and the first generation or so show many bacilli, and forms intermediate between bacilli and cocci. Also, the elimination of all bacillus forms from such a culture often requires a good many transplantations. Further, individual strains (cultures from different sources) of the organism vary considerably in their amenability to these transformations.

*Gram*.—Gram-positive staining properties were developed in limed serum mixtures, and were also manifested whenever the complete development of the organism took place, i. e., when polar bodies were formed. In transplants from a medium conducing to the development of Gram-positiveness to one tending to make the organism Gram-negative, or vice versa, it is to be noted that a few organisms, retaining the parent's characteristic, usually appeared in at least the first transplant. But when the new conditions were continued in further generations such "reversions" tended to disappear.

*Ferment*.—Liquefaction of the Loeffler seemed to depend upon whether the organisms in a given culture reached the sporulating stage in large numbers or not; so it seems that sporulation frees the ferments that cause this phenomenon. Note that whenever the Gram-negative, nonsporulating form persisted no liquefaction appeared. Difficulty was experienced, as has been noted, in causing Culture 5 to liquefy Loeffler, and it was only by replanting, on Loeffler, organisms from a pellicle which had formed on a bouillon culture that this was finally effected.

*Gas Production* was noted in the case of Culture 1 only. When first isolated, the organism of this culture failed to produce gas on several trials. Later a small amount of gas was produced in bouillon containing 1% dextrose. No gas was produced by the organisms of the other cultures tested, although it is believed that all could be got to effect its production, by more extended effort, just as was the case with Culture 1.

*Indol*.—After a growth in 1% peptone water for six days, the nitrosoindol test (Salkowski) was applied with the following results:

Culture 1	Faint pink ring.
Culture 2	None.
Culture 3	Faint pink ring.
Culture 4	Distinct pink ring.
Culture 5	None.
Culture 6	None.

Indol production (detectable by this test) is therefore, probably, an unstable characteristic of the organism and, I believe, could be obtained with any culture.

*Reaction of Cultures.*—Replants of six cultures were made in two sets of tubes of bouillon colored with litmus, one set slightly acid and the other faintly alkaline. Growth was obtained in both sets and while no change in color in the acid set was observed the blue color of the alkaline set had changed, by the end of a week, to a greenish. We may, therefore, conclude that the organism produces acid in bouillon cultures.

*Motility.*—Original plants in fluid media of high serum percentage show no motility, unless a tumbling motion of individual "coccoids" may be denominated such. Replants, however, in fluid media not containing serum, show a motility which varies with the culture—sometimes sluggish or entirely absent, but commonly very active. A replant of a motile culture in serum media commonly gives a non-motile organism.

Smears from motile cultures showed that the organism has a single short terminal flagellum (Van Ermengem's stain).

*Filterability.*—Cultures (from eight sources), in lined-hydrocele-bouillon (2 days' growth) and three day liquefying cultures on Loeffler were passed through Berkefeld filter No. V. Plants made from the sixteen filtrates gave profuse growths similar to the original cultures.

*Temperature of Growth.*—Replants of cultures from eight sources (including western) were planted on plain Loeffler and incubated at 75° F. When it was found that good growth was obtained, a similar set was incubated for 48 hours at 70° F. ("summer heat"). Six of these cultures showed a profuse development, and two only a faint growth. First generation replants from the latter gave a profuse growth under the same conditions within a second 48 hours.

*Cultivation in Milk and Resistance to Pasteurization.*—Cultures of the eight "strains" mentioned were sown in two sets of tubes containing sterilized milk of the market variety (24 hours old, slightly acid to litmus) and one set incubated at 98° and the other at 70° F. for 48 hours. At the end of this period all cultures were swarming with minute coccoid bodies, and yet no apparent change had taken place in the milk, with the exception of Culture 1, grown at 98°, which had coagulated. Both sets of cultures were heated in a water bath for 30 minutes at 142° to 145° F. and replants from all then made. Good growths resulted in all instances. This experiment was repeated again, only allowing 24 hours for the development of the organisms in the milk. At the end of this period organisms were plentiful in all the cultures, but much fewer in those grown at 70° than in the corresponding tubes incubated twice the time (48 hours). No coagulation took place with Culture 1. All cultures survived the pasteurization process (30 minutes, 142° to 145° F.) and gave profuse growths on replants thereafter.

## ANIMAL TESTS.—

*Rabbits.*—Among fourteen rabbits of a series (each injected in the ear vein with from  $\frac{1}{4}$  to 1 c.c. of a fluid poliomyelitis culture—bacillus form) two developed a flaccid paralysis of one hind leg, noticeable on the third day, and one a partial paralysis, showing as weakness of hind legs. No other symptoms, except general weakness, were noticeable. The last mentioned died on the fifth day, and two others were killed, and autopsies made on the three. Meninges of brain and cord, and portions of the gray matter beneath, of the three were markedly congested, and microscopical examination showed areas of infiltration around blood vessels.

One rabbit died without manifest symptoms, on the second day following inoculation, and autopsy showed the same meningeal and gray matter engorgement.

Ten rabbits showed no definite symptoms, but three of them, killed on the fourth day following injection, showed considerable congestion of brain and cord.

*Cats.*—Similar inoculations made into the saphenous veins of young cats gave results as follows:

Two died on the fourth, and one on the sixth day, after having shown extreme prostration and having refused all food. During the four days of illness all manifested a nasal discharge, and sneezed very frequently.

After showing similar symptoms, eight cats recovered within periods ranging from two days to two weeks.

One cat, on the second day, developed marked paralysis of the back muscles and a weakness of one hind leg. Two others showed a transient paralysis manifested by distinct "weakness" of hind legs. The last three animals also showed a nasal discharge, with accompanying sneezing.

All of the cats were given from 0.6 to 2 c.c. of a fluid culture.

Autopsies on the three cats that died, and on five that were killed within two weeks following injections, showed similar lesions to those found in the case of the rabbits, in one, however, small cerebral hemorrhages had taken place.

*Dogs.*—Swabs soaked with a fluid culture were rubbed over the nasal mucosa of two young dogs with the result that from the second day for about two weeks one manifested a severe and the other a mild catarrh. The first showed considerable discharge but the latter only frequent sneezing.

Another (a young fox terrier) inoculated in the ear vein with 1 c.c. of a fluid culture was made very ill for about a week with symptoms of prostration, fever, and frequent sneezing. At the end of this time, as he apparently was recovering, a second dose of double the quantity was given in the same manner. The next day he was apparently quite sick but showed no catarrhal symptoms. On the second day he was very weak in the hind legs and, towards evening, I noticed that he carried his right hind leg, which was evidently partially paralyzed. On the third day his left fore leg was likewise affected and, while he could use it in standing, it was very weak. This dog's bladder was also involved in the paralysis, since urine dribbled when he moved, or when his abdomen was struck.

Another young dog, inoculated with 3 c.c. of the same culture, died on the fourth day, after great prostration and some catarrhal manifestations.

One adult dog, inoculated with 1 c.c. of the culture, showed no symptoms beyond apparent fever and anorexia lasting about 24 hours.

Two puppies, four weeks old, each intravenously inoculated with 1 c.c. of the culture developed within 24 hours extreme prostration and marked catarrh which within three days showed as a marked nasal discharge. Both dogs died on the fifth day. The three dogs that died and the other four, which were chloroformed a week after receiving their dose of the culture, were autopsied.

The two dogs, of which the nasal mucosa was swabbed with a culture, showed no lesions beyond a congestion of the mucous membrane mentioned.

The paralyzed dog showed marked congestion and infiltration of both brain and spinal cord, together with a number of small hemorrhages into the gray matter of each.

The unaffected adult dog showed a congestion of the meningeal vessels, principally those of the brain.

The lesions in the three dogs that died were more marked: All had extreme congestion of the meningeal vessels of both cord and brain, and a marked infiltration of the adjacent gray matter. Small hemorrhages into the gray matter and from the meningeal vessels were common in the three dogs.

*Lesions Outside Nervous System.*—Autopsies of all the animals—rabbits, cats, guinea pigs, and dogs—that died as a result of the inoculations disclosed general hemorrhagic tendencies especially marked in the subcutaneous vessels. One such cat, two rabbits, one guinea pig and a dog, of the same category also manifested considerable congestion of the kidneys, and in the case of the last mentioned cat both spleen and liver were markedly swollen. The nasal mucosa of nearly all of the animals was congested in varying degree. One rabbit also showed a severe general congestion of both lungs.

From the brains of all of the animals described, that died or were killed, the organism was recovered in pure culture.

The similarity of the symptoms of experimental poliomyelitis in animals, as evidenced in the preceding experiments, and those of others, to the disease known as distemper is most interesting.

No inoculations (except of the one rabbit described) were made directly into the central nervous system, since it was felt that paralysis might follow such operations even in the case of organisms which could never naturally affect it.

*Relationship of Poliomyelitis and Distemper.*—Among dogs suffering from distemper paralysis develops in a small percentage of the animals.\* For in-

\*In connection with the frequency and form of nerve center involvement in distemper of dogs, the following quotations are of interest:

"In a few cases the disease leaves after it a permanent paresis of the hind-quarters and a vesicorectal paralysis. We have several times noticed paralysis of the tongue." (Friedberger and Frohner: Path. and Ther. of the Domestic Animals, ii, 35.)

The same authority says that "cerebral edema" is usual, and also "slight edema of lumbar region" of the cord. In "acute paralysis," accompanying distemper in dogs, an exudate is found around the blood vessels of the nerve center and a degeneration of the latter. In "chronic paralysis," "circumscribed interstitial myelitis, with partial atrophy of the nerve substance," is the rule (Ibid., 354).

"Paralysis may follow the convulsions (in distemper) or it may come on simultaneously with them. It seldom occurs at the beginning of the disease. It may be confined to certain groups of muscles, as those of a limb, the whole of the hind-quarters, or even involve the entire body in the form of paresis, especially of the motor nerves, combined with excessive muscular weakness.

"Usually the brain is the seat of edema. In some cases the brain substance, especially the walls of

stance, during six months, ending Nov. 30, 1916, the pay clinic for dogs of the A.S.P.C.A. (in New York City) treated 3,055 dogs, and 506—16.5 per cent—of these are recorded in the clinic register as suffering from distemper. Among these distemper cases only about 5 per cent showed paralytic symptoms or signs of nerve center involvement.

It is well known that cats suffer from distemper due to infection by the same organism that causes the disease of dogs, but a cat so affected only occasionally shows paralysis.†

The following, partly inadvertent, experiment is interpreted as strongly indicating the identity of the two organisms (poliomyelitis and distemper). In injecting a cat (intravenously) with a culture of the organism, and wishing to give a second syringeful of the material, I detached the needle, leaving it in the vein, and refilled the syringe, then returned it to its connection with the needle. Resistance was, however, met to the flow through the needle (owing probably to a blood clot) and when I added extra pressure to the piston some of the culture squirted out around the needle-syringe joint, a drop reaching one of my eyes. It was carefully washed out with a solution of a silver salt and I thought no more about it. About 72 hours later I felt a slight soreness of the throat and some signs of digestive disturbance, including a slight headache. Temperature was not taken, but if it was elevated at all, it must have been very slightly. I went ahead with my usual routine, never suspecting anything but one of the every day indispositions with which everyone is occasionally troubled, although I afterwards realized that I had felt very unusually nervous, all day. After I got to bed I could not, as I, as a rule, invariably do, get to sleep at once, but had to sit up repeatedly owing to an unexplainable feeling that I could not breathe. There was not the slightest interference with control of any respiratory act nor of any muscle. Through the night I slept very little, owing to a repetition of this uneasy sensation. The next day I felt fairly well, though slightly weak, which continued to be the case for several days.

The following morning I began to suspect that the cause of the sensation might be a poliomyelitis-organism pharyngitis, and determined to investigate. The post-pharyngeal mucous glands appeared markedly swollen, and scrapings disclosed a great many spear-head shaped "streptococci-like" organisms, which subsequently gave developments on cultivation identical with those of the culture I had used on the cat.

The same day, four platinum-wire loopfuls of mucus from my pharynx were mixed in 1½ c.c. of saline solution, and 1 c.c. injected, intracerebrally into a rabbit, and ½ c.c. intraperitoneally into a young guinea pig. The rabbit developed paralytic symptoms within 24 hours, affecting principally the adductor muscles of his legs, but as he fell about considerably I thought that his sense of equilibrium was affected (Fig. 5). This rabbit appeared to be recovering for some days, but finally died, and the autopsy showed marked engorgement of the

the vessels, is infiltrated with leucocytes. Changes in the spinal cord, which are not well marked, consist chiefly of anemia and slight edema, especially in the lumbar region." In acute paralysis, there are changes in the walls of vessels, with an exudate along the vessels and in the interstitial tissue of the gray matter of the spinal cord. In chronic distemper there is a localized interstitial myelitis with partial atrophy of the cord: (Moore: *The Pathology and Differential Diagnosis of Diseases of Animals*, 343).

†Ibid.

brain and cord, and, microscopically, much round cell infiltration and a considerable number of organisms that upon cultivation proved to be identical with those of the original culture.

The guinea pig died on the fourth day, and the postmortem showed congestion of the meningeal vessels of the cord. Those of the brain were extremely congested, and in many places had given rise to small hemorrhages. Everywhere in the substance of the brain the same engorgement of the blood vessels was apparent, and many extravasations of blood were found. Round cell infiltration of the adjoining brain matter was distinct. Cultures, from the brain and cord, gave the organism of the culture originally used.

The above mentioned guinea pig had been kept in the same cage with another of the same age, an adult guinea pig and a young rabbit. Five days after the death of the first guinea pig I found the companion guinea pigs dead, and



Fig. 5.—Rabbit intracerebrally inoculated with 1 c.c. of saline containing two platinum loopfuls of mucus from the throat of the experimenter accidentally infected with Culture 4. Photograph taken three days before death.

the rabbit sick. Autopsies of the two guinea pigs disclosed extreme congestion of the vessels of the brains and cords, with small hemorrhagic extravasations here and there, upon and in the brain tissue, and accompanying round cell infiltration.

An illustration herewith shows the congestion of the meningeal vessels of the second young guinea pig (the contact case).

The contact rabbit died 24 hours later and a similar involvement of the nervous system, without other apparent lesion, was found on autopsy. The brains and cords of these three animals gave the organism of the culture originally used on the cat.

*Feeding Experiments.*—The carcasses of the two infected-by-contact guinea pigs were fed to two cats, one of which developed the symptoms of distemper (catarrh and prostration) with the apparently identical organism in the discharge. The attack took four days to develop and lasted about a week.

Two cats fed on 2 c.c., and 3 c.c., respectively, of cultures (grown in milk) developed a copious diarrhea which lasted seven and ten days, respectively.

*The Organism from Naturally Acquired Distemper in Animals.*—Something should be said here in regard to the organism causing distemper in animals, and its characteristics:

I find it stated, generally, in textbooks and the other literature, that all (or most) of the domestic animals suffer from infectious processes due to organisms which are at least allied to the bacillus causing the distemper of dogs and cats. Characteristics common to all of the organisms so operative are described as follows:\*

"Characteristics common to the pasteurilla group.—Organisms of the pasteurilla group are nonmotile coccobacilli, Gram-negative, very pleomorphic, and staining more deeply at the ends than in the center. They do not form spores, do not liquefy gelatin nor coagulate milk, they give no visible growth on potato, are primarily aerobic, but can be grown anaerobically. They give rise in culture to a peculiar and characteristic odor.

"According to Lignieres, these organisms do not produce indol in culture, but old cultures of the bacillus of fowl cholera (the avian pasteurilla) certainly contain indol."

Besson, the author of the textbook quoted, describes the organisms of this group under the general term "Pasteurellas" and, in regard to the group as a whole, writes:†

"As Nocard held, the organisms isolated from different animal species must be regarded as varieties of the same bacillus, and the conclusion arrived at is in short this: that there is one Pasteurella, which can pass from one animal species to another, and which by adaptation in one species can produce a disease peculiar to that species."

Last fall I obtained cultures from three dogs suffering from canine distemper, in order to compare the organism with that from poliomyelitis, with the following results:

Mucus from the nares and nasal discharges of all three dogs showed great numbers of small bipolar bacilli which varied considerably in size, and in their response to the Gram stain—in the same smear some appearing Gram-positive, some Gram-negative, and some intermediate (not positively either positive or negative). Cultivation of these organisms, by the methods detailed in connection with the poliomyelitis cultures, gave growths of an organism which corresponded in all particulars (not detailed as space would hardly permit) with the poliomyelitis bacilli. There were slight differences, to be sure, but only similar ones to those between poliomyelitis cultures of different source.

However, the characteristics of the distemper bacilli isolated did not correspond in some important particulars with those given for the class in the quotation from Besson: they could be grown to show motility‡ and also involution forms resembling spores. In these particulars they varied just as did the poliomyelitis bacilli; in accordance with the method of cultivation.

\*English Edition of Besson's "Practical Bacteriology, Microbiology, and Serum Therapy," p. 447.

†Ibid., 446.

‡McGowan (Jour. Path. and Bacteriol., xv, 372) stated that the bacillus of dog distemper was "slightly motile."

The culture denominated Distemper I, mentioned in this article, was obtained from a hound under the care of Dr. Little, Veterinarian in charge of the A.S.P.C.A. clinic, previously mentioned.

Agglutination tests of this bacillus, against specific antipoliomyelitis sera, and against the blood of poliomyelitis convalescents are given in the accompanying tables. Results vary much as with poliomyelitis cultures.

TABLE I.  
SUMMARY OF AGGLUTINATION TESTS.\*

BLOOD SPECIMENS.				
<i>In dilutions of 1:40—</i>				
11 blood specimens completely agglutinated one or more of the six cultures.				
In this dilution:				
7	specimens completely agglutinated	1	culture each.	
3	" " " "	2	cultures "	
1	" " " "	3	" "	
<i>In dilutions of 1:20—</i>				
26 blood specimens completely agglutinated one or more of the six cultures.				
In this dilution:				
14	specimens completely agglutinated	1	culture each.	
8	" " " "	2	cultures "	
2	" " " "	3	" "	
1	" " " "	4	" "	
1	" " " "	5	" "	
<i>In dilutions of 1:10—</i>				
37 blood specimens completely agglutinated one or more of the six cultures.				
In this dilution:				
9	specimens completely agglutinated	1	culture each.	
12	" " " "	2	cultures "	
6	" " " "	3	" "	
5	" " " "	4	" "	
3	" " " "	5	" "	
2	" " " "	6	" "	
CULTURES.				
Number		Dilution 1:10	1:20	1:40
1	Times completely agglutinated	11	4	1
2	" " "	24	12	4
3	" " "	10	4	1
4	" " "	20	9	1
5	" " "	22	14	8
6	" " "	11	2	1

\*Thirteen (13) specimens failed to completely agglutinate any of the cultures. It is noteworthy that all the acute cases (6) were among this number.

Serum tested was from three cats, injected each three times with increasing doses of cultures. Serum A was made with Culture 1; Serum B, with Culture 3; and Serum C, with Culture 4 (Table II).

SEROLOGY.—Agglutination Tests.—Table I contains a summary of 1500 agglutination tests made with the blood of fifty children, stricken with poliomyelitis in the recent epidemic in New York City. Thirty-six of the children were in the Seaside Hospital, Brooklyn, nine in the Riverside Hospital (for acute cases), one in the Long Island College Hospital and four under the care of various Brooklyn physicians.

In all, except one case, dried blood was used in making the required dilutions, which were standardized by color. The dilutions are recorded in parts of blood



(not serum) so that 1 to 10 agglutination represents 1 part of blood in 10 parts of saline and culture.

In glancing over the table it will be seen that no blood completely agglutinated in dilutions greater than 1 to 40, and, we find that in this dilution but 11 of the 50 specimens were effective. In dilutions of 1 to 20, 26 specimens completely agglutinated one or more cultures, and in 1 to 10, 37, while 13 failed to completely agglutinate any culture.

In Table II are recorded agglutination tests made with sera from cats which

TABLE II.  
ANTIPOLIOMYELITIS SERUM AGGLUTINATION TESTS.  
(Tests recorded after incubation for one hour at 98° F.)

Serum	A							B							C						
Culture	1	2	3	4	5	6	D	1	2	3	4	5	6	D	1	2	3	4	5	6	D
Dilution of serum																					
1:10	C	C	C	O	C	C	C	C	C	C	S	C	C	C	C	C	S	C	S	C	C
1:20	C	P	C		C	C	C	C	C	C	O	C	C	C	C	C	S	C	S	C	C
1:40	C	S	C		P	C	P	C	C	C		P	C	S	C	P	O	C	O	S	O
1:80	C	O	S		O	C	S	C	C	C		S	C	O	P	S		C		O	
1:160	C		O			P	O	C	P	C		O	P		S	O		C			
1:200	C				O			P	O	C			O		O			P			
1:320	C							O		P								S			
1:400	C									S								O			
1:500	C									O											
1:600	C																				
1:700	P																				
1:800	P																				
1:900	S																				
1:1000	S																				

Key: Cultures 1 to 6 are the same as those so numbered in Table I. Culture D is of distemper bacillus 1. O, S, P, C denote: no, slight, partial, and complete agglutination, respectively.

TABLE III.  
POLIOMYELITIS BLOOD AGGLUTINATION OF DISTEMPER BACILLUS 1.  
(Tests recorded after incubation for one hour at 98° F.)

Case No.	35	36	37	38	39	40	41	42	43	44	45
Dilution 1:10	O	P	C	C	P	P	O	P	C	O	P
1:20		S	C	C	S	S		S	P		O
1:40		O	P	P	O	O		O	S		
1:80			S	S					O		
1:160			O	O							

Key: O, S, P, C, denote: no, slight, partial, and complete agglutination, respectively.

had been immunized against poliomyelitis cultures to such a degree that (Serum I) ten times the ascertained usually fatal dose failed to affect. From this table we see that one culture was completely agglutinated in dilutions of 1 to 600, which shows that the poliomyelitis organism is quite susceptible to this process.

Table III (referred to elsewhere) shows the agglutination of a distemper bacillus by poliomyelitis blood.

In Table IV are recorded agglutination tests made with sera of adults and children who had never had poliomyelitis. The minimum strength in which any

of these bloods completely agglutinated any of the six cultures was 1 to 20, 4 out of 10 specimens agglutinating in this strength.

From these facts we can at least compare the strength of naturally acquired immunity in man and that which may be induced by vaccination, as measured by the agglutination test, a comparison which is seen to promise well in reference to the possible effectiveness of antipoliomyelitis sera, since serum A was at least 15 times as powerful as the "strongest" blood from a convalescent child.

TABLE IV.  
NORMAL HUMAN BLOOD AGGLUTINATION.  
(Tests recorded after one hour at 98° F.)

Number	1A	2A	3A	4A	5A	1C	2C	3C	4C	5C
Poliomyelitis										
Culture 1										
1:10	O	S	S	O	P	O	O	P	O	O
1:20		O	O		S			S		
1:40					O			O		
1:80										
Culture 2										
1:10	C	C	C	C	C	O	S	C	O	O
1:20	P	C	C	C	P		O	C		
1:40	S	P	P	P	S			P		
1:80	O	S	S	S	O			S		
Culture 3										
1:10	O	C	P	O	S	O	S	C	O	O
1:20		C	S		O		O	P		
1:40		P	O					S		
1:80		S						O		
Culture 4										
1:10	P	C	S	C	S	P	P	C	O	O
1:20	S	P	O	C	O	S	S	C		
1:40	O	S		P		O	O	P		
1:80		O		S				S		
Culture 5										
1:10	O	S	P	C	S	O	P	C	O	O
1:20		O	S	C	O		S	C		
1:40				P			O	P		
1:80				S				S		
Culture 6										
1:10	O	O	O	O	S	O	O	P	O	O
1:20					O			S		
1:40								O		
1:80										

Key: Same as for Table III. Specimens are numbered in two series—those from adults, comprising the A series, those from children the C series. In the case of the adults, serum was used; in the case of the children, dried blood.

As only three inoculations of the animal were made in producing this serum, it may be surmised that a much more powerfully agglutinative serum might easily be developed.

In reference to the normal blood reactions, recorded in Table IV it should be mentioned that 2C and 3C are from two sisters, residents of Brooklyn, and exposed to the recent epidemic; and that 4C and 5C are from children in other communities not visited by the disease. Case 1C is from my own son, a boy of 16 years of age, and is particularly interesting in that Culture 4, the only one

agglutinated at all, is the same as that of which a drop is recorded as having accidentally entered my eye.

The reactions given by the adult blood specimens seem to be about such as we might expect from known immunes, as adults so generally are. All completely agglutinated at least one of the six cultures in dilutions of 1 to 10.

*Specific Agglutinability of Individual Cultures.*—In the tests with convalescent blood there are so many "overlappings," as it were, in which two or more blood specimens agglutinate the same and also different cultures, that it would be difficult to separate the cases into groups in accordance with the cultures agglutinated. However, with the specific antipoliomyelitis sera tested (in Table II) we find a markedly specific agglutination, as with serum A and its antigen, Culture 1. Something of the same is seen in the case of the other two sera and the cultures used to produce them.

Note should particularly be made of the fact that while serum A would not agglutinate Culture 4, the serum made with this latter (4)—Serum C—strongly agglutinated the culture of Serum A (1). Also that Sera B and C, respectively, only slightly agglutinated (1:10) the culture used to produce the other.

These are interesting, but not easily explicable, facts which, however, strongly point to the probability that in producing a serum for the treatment of poliomyelitis, polyvalence will be necessary.

#### ACUTE CASE OF POLIOMYELITIS TREATED WITH SPECIFIC SERUM.

While visiting Riverside Hospital to get nine of the blood specimens recorded in the preceding, (Table I) the resident physician in charge, J. W. Crawford, asked me if I knew where he could obtain some serum from an immune person, as the physician of R. Z., a 16 year old boy specially wished to have it tried, as he feared the patient otherwise had no chance of recovery. I told him of the cat serum that I had prepared and, after the patient's physician had been consulted and had urged that it be tried, this was done.

The cat sera were first tested for any possible agglutinative and hemolytic action which they might have on the patient's corpuscles, and Dr. Crawford then gave him intravenously a mixture of Sera A, B, and C, about 35 c.c. in all.

The patient had been paraplegic and had suffered from bowel and bladder paralysis since his attack began, six days before. His temperature had been constantly about 103° F., and he was very restless and complained of great pain along his spine. He was coughing considerably and a lung complication was believed imminent.

No effect was noticeable from the serum until about 24 hours after its administration, when the patient's bowels and bladder emptied themselves for the first time since his illness began, six days before; and the pain in his spine was markedly relieved. Relief in these particulars continued, but the boy's lung complication grew worse, and he died, three days later, of pneumonia of the left lung. This case was one of the last of the epidemic, and no other opportunity has since presented for further trial of the sera.

## SOME EPIDEMIOLOGICAL FACTS IN RELATION TO THE BACTERIOLOGY.

The almost exclusive prevalence of poliomyelitis in summer has been a subject of much interest, and the marked subsidence of epidemics at the commencement of the cooler fall temperatures has been the cause of much speculation. Therefore, the fact, demonstrated in the foregoing, that the organism multiplies readily at "summer heat" (70° F.) is interesting, and, I think, very enlightening as to the cause of summer epidemics. Especially important seems the fact that the poliomyelitis organism grows well at summer temperatures in market milk, and that pasteurization does not kill it.

The literature is full of evidence as to how markedly epidemics of poliomyelitis are affected by the outside temperature, and, as a recent example, it is interesting to note the mean temperature, and the number of cases of poliomyelitis reported, in New York City, during each of the warm months of 1916:

MONTH.	MEAN TEMPERATURE.	CASES.
May	59.3°	29
June	64.2°	756
July	73.8° (over summer heat)	3863
August	73.6° (over summer heat)	3306
September	66.0°	780
October	57.2°	193

During the course of the epidemic that visited Washington, D. C., in 1910, the temperature varied between 69.7° and 77.6° according to the report of the special commission of investigation.

Recently I have gone over most of the literature on the epidemiology of poliomyelitis, covering more than 50,000 cases, and have noted the evidence contained therein that few or no babies develop the malady while exclusively breast fed. However, definite statements concerning the manner in which the affected children have been fed are few, and to such evidence we have to add that of the rarity of the occurrence of the disease in babies under one year of age—who alone would be likely to be so fed—and its extreme prevalence (in at least 90 per cent of all cases) in children under ten.

I have some of the statements found in the literature, bearing on this question: New York City (1907)—"121 cases exclusively breast fed among 283 under 2 years." Massachusetts (1907)—"None of 7 cases under 1 year were exclusively breast fed." Westphalia (1909)—"Considerable number of children at the breast were affected." Massachusetts (1909)—"No case among exclusively breast fed." Iowa (1910)—"Two cases exclusively breast fed among 47 considered." Cincinnati (1911)—"Fifteen cases exclusively breast fed among 83 considered." Buffalo (1912)—"Twelve cases exclusively breast fed among 40 considered."

How the age incidence of the malady indicates that few or no exclusively breast fed infants are affected is illustrated by the figures of the recent epidemic in New York City:

Total cases	9,023
Under 1 year	11%
1 to 5 years	68%
5 to 10 years	16%
10 to 16 years	2%
Over 16 years	3%

Note that while 95% of all the cases were under ten years of age, only 11% were under one year!

The bacteriological relationship of poliomyelitis of man to distemper of the domestic animals is illustrated, epidemiologically, in Table V, which I have compiled from the literature:

TABLE V.

## ACUTE PARALYTIC DISEASE AMONG DOMESTIC ANIMALS COINCIDENT WITH EPIDEMIC POLIOMYELITIS.

POLIOMYELITIS EPIDEMIC.	ANNOTATION.	AUTHORITY.
Vermont (1894)	"Horses, dogs and fowls affected with paralytic disease."	Caverley: Jour. A. M. A., 1896, xxvi, 1.
Sweden (1905)	"Paralysis among dogs and other animals."	Wickmann.*
Dubois, Pa. (1907)	"Paralysis among pigs and chickens."	Free.*
Wisconsin (1907)	"Paralysis among colts, sheep, cats, ducks, and many other animals."	Manning.*
Oceana Co., Michigan (1907)	"Paralytic disease of chickens."	Griffin: Jour. Mich. Med. Soc., Feb., 1908.
Rhenish Westphalia, Germany (1909)	"Great mortality among chickens."	Römer: On Epidem, Kinderlah, Berlin, 1911.
Massachusetts (1909)	"Paralytic disease prevalent among animals."	Lovett: Month. Bull. Mass. St. Bd. of H., June, 1910.
Minnesota (1909)	"Colts, paralysis among."	Hill.*
California (1910)	"Paralysis among colts, dogs, cats, and chickens."	Snow.*
Washington, D. C. (1910)	"Paralytic disease appeared among ducks and chickens just prior to the outbreak."	Special Committee of Investigation.
Iowa (1910)	"Paralysis among cats, hogs, and chickens."	Bierring.*
California (1910-11)	"Paralytic disease coincident among domestic animals."	Gundrum: Cal. State Jour. Med., May, 1913.
Sao Paulo, Brazil (1910-11)	"1000 horses and 4000 cattle died with 'symptoms' of rabies."	Carina.*
Cincinnati (1911)	"Paralytic disease coincident among domestic animals."	Frost: Hyg. Lab. Bull., No. 90.
Indiana (1911)	"18 paralyzed animals associated with 102 cases of poliomyelitis."	King.*
Ohio-Kentucky (1911)	"Paralysis among chickens."	Batte.*
Alaska (1913)	"Outbreak was preceded by a great epidemic of distemper among the dogs."	Pierson: Jour. A. M. A., Feb. 28, 1914.

\*Quoted by Frauenthal and Manning in their book on poliomyelitis, "Infantile Paralysis."

## CONCLUSIONS.

It is demonstrated that the organism isolated from the nerve centers of cases of poliomyelitis (including the "streptococcus" described by various observers) is a pleomorphic bacillus of the distemper group, which varies in characteristics much as the various, supposedly different, members of the group do from one another; that this poliomyelitis bacillus could cause paralysis in cats, dogs, rabbits, and guinea pigs, and that an accidental passage of a culture through man gave rise to abortive symptoms of the malady; that after this last named passage it could produce paralysis in a rabbit, and a contagious infection of guinea pigs,

with nerve center lesions; and, finally, that from the guinea pigs it could produce distemper in cats.

Further it is shown that the organism is saprophytic and grows well in milk at "summer heat;" and that it resists the pasteurization process, while contained therein. Also, that it forms "spores," and is a "filter passer."

It seems very probable that, while contact cases of poliomyelitis may occur, either by direct transmission of the germ from animal to man, or from man to man, the great mass of cases which comprise epidemics are caused by milk borne contagion, and, furthermore, we shall have to regard our cows as possible "carriers" of the infection. It is, of course, evident that infection could get into milk from other animals, as the cat and dog, or even from a human carrier (as was supposed to have occurred in a series of cases reported by John C. Dingman, of Spring Valley, N. Y., in the December number of the *New York State Journal of Medicine*.)

Special thanks are due to Anna Williams, Assistant Director of the Research Laboratory, New York City Department of Health, for pathologic material; J. W. Crawford, Acting Resident Physician, Riverside Hospital, New York City, for blood specimens and trial of specific serum; Walter Truslow, Chief Attending Orthopedist of the Seaside Hospital, Brooklyn, N. Y., for blood specimens; and Dr. Little, Veterinarian in charge, A. S. P. C. A. dog clinic, for cultures, etc.

## NEWER CONCEPTIONS OF DEMENTIA PRÆCOX BASED ON UNRECOGNIZED WORK\*

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JUST as our knowledge of any given subject comes to being out of many little nebulous masses, so we have gained a certain amount of insight into the nature of dementia præcox. And as the science of any subject owes its accurate development to the work of the laboratory, so our present knowledge of dementia præcox has been built up from the many little nebulous masses, which have developed in the laboratory but which have developed into a concrete whole; paradoxical as the idea may seem. And yet, this disease form which makes up nearly a quarter of all the cases admitted to hospitals for the insane and which accounts, more than any other single factor, for the necessity of building more of such institutions, has been neglected from the only side which can possibly give us hope of combating its spread, the laboratory side. Its treatment has been largely custodial, symptomatic, not to forget recent developments in industrial teaching and in social service work.

Laboratories deal with technical methods and for this reason a discussion of our present knowledge of dementia præcox or a presentation of newer conceptions of dementia præcox from the laboratory point of view must be more or less intimately linked with a discussion of the methods used in obtaining the knowledge.

The institution of exact methods begins with the investigation of the cause of death by means of the postmortem examination. The earliest investigation of a case of dementia præcox that I have been able to find, where the brain and organs were examined and the examination published, was in 1844 by Alquié (quoted by Cramer<sup>1</sup> in 1896). The chief finding of interest to us is that the gray matter of the brain was injected. This observation I take to be of the utmost importance, not only because it was a true observation, doubtless, but because it is a frequent finding even today, though not confined to dementia præcox, of course. The method of observation is also important and the one most generally used in medicine. It is called the gross method or the macroscopic examination. Using this method, much valuable information has been obtained concerning the nature of dementia præcox.

Jehn,<sup>2</sup> in 1877, speaking of acute delirium, and some of these cases certainly look like dementia præcox mentally, remarks on a high grade of vascularity found; as did Alquié. There is hyperemia, clouding of the pia, reddening of the cortex, increased cerebrospinal fluid, and edema of the brain. There are changes in the other organs and the hemoglobin comes out of the blood. L. Meyer (1857), speaking of "acute fatal hysteria" mentions extensive venous hyperemia (Cramer<sup>1</sup>). Pauly<sup>3</sup> (1869) in a dissertation at Bonn, mentions edema resulting from hyperemia. Schüle<sup>4</sup> (1879) separates two types of acute delirium—one showing hyperemia of the pia, a few with serous edema, especially of the frontal

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lobes, some with milky, diffuse or punctiform clouding and reddening of the cortical surface of the meninges—the other group showing nothing in the meninges, but a venous hyperemia in the brain and an increase in the cerebrospinal and ventricular fluid. Rezzonico<sup>5</sup> (1884) gives the history of a male of forty-seven years of age who died after eleven days of excitement. There were no external signs of disease or injury, but the cortex and white matter were remarkably hyperemic. Similar observations were made by Ball<sup>6</sup> (1885) and Holsti<sup>7</sup> (1884). Thickening of the meninges without exudate is frequently seen, as noted by Klippel and Lhermitte (quoted by Zingerle<sup>8</sup>), Obregia,<sup>9</sup> Schütz<sup>10</sup> (1909), Lukacs<sup>11</sup> (1908), Marchand,<sup>12</sup> Goldstein,<sup>13</sup> and Weber<sup>14</sup> (1899). So hyperemia of the brain, thickening and adhesions of the meninges, were the observations of the day in cases which we can recognize today as being those of dementia præcox and of the infections or intoxications and these findings were linked with the interests of the day such as intracranial circulation, pressure, and stasis. Such were also the findings of Kahibaum<sup>15</sup> in his monumental work differentiating katatonia, or tension insanity, as he called it.

The gross method was not exhausted with these observations but turned in new directions after many years. Examination of the convolutions came next with the claim that there was atrophy (Klippel and Lhermitte,<sup>16</sup> 1907), that there was decrease in size of the white matter and of the cortex (Obregia, 1906—Zimmermann,<sup>17</sup> 1907), that there was hemiatrophy and lobar atrophy of the cerebellum (French writers and later Morse and Taft<sup>18</sup>), that there was convolutional asymmetry (Mondio, quoted by Omorokow<sup>19</sup>).

Another form which the gross method took was the investigation of the relation existing between brain weight and cranial capacity. This was first made practicable by Reichardt<sup>20</sup> (1907-1911) who showed that there was little difference between the weight of the brain and the capacity of the cranium in certain cases of katatonia, dying suddenly, with or without convulsions. The same was true in some cases of epilepsy, the difference being 0%. Cases described by Nonne<sup>21</sup> as "pseudotumor cerebri" (1904) are of the same sort, and their histology has been described in detail by Rosental<sup>22</sup> (1911). These are cases which give evidence of tumor clinically, but prove the diagnosis to be wrong by cure or autopsy. Such a case was published by Dreyfus<sup>23</sup> (1907) from the Basle Psychiatric Clinic. The patient was a male dementia præcox of twenty-nine at onset, who died suddenly at 32 years of age. The brain was stained with several stains, in sections taken from eight different areas, and no changes were found but the cord was large for the spinal canal and the brain was 140 grams too heavy for the cranial capacity. Death was due to acute brain swelling.

The interval between these two great periods of research with the gross methods was taken up with the study of the minute details of brain pathology by means of the microscope and the newly discovered color chemistry. Most of the work on dementia præcox, as in other branches of pathology, has been done with the methods of finer histology, neglecting the more logical ones of photographing the gross changes observed so that they might be preserved for comparison in large numbers of cases from all parts of the world; neglecting the fact that study of the frontal section in series might yield something and this to be photographed; neglecting the fact that the whole brain microtome can give



valuable information in the psychoses; and going directly to the finer histology with the attempt to get the section ever thinner.

The results obtained over the years have been very much at variance and often appeared to be extremely contradictory, but the time has come when a certain uniformity can be put upon them and they can be understood.

To unify the large amount of material which has accumulated and at the same time to show that the changes found are very much alike, I have selected cases from the following groups and have arranged the results under the dementia præcox group, the confusional and delirious group, a single case of "pseudotumor," and the general work on the nervous elements in the psychoses.

In 1896 the findings in the acute case of the paranoia group were given by Cramer.<sup>1</sup> A male of twenty-four, duration seven days, had shown albumin and fatty casts. Temperature was 40.5° C., pulse 132, respiration 48. Autopsy was performed 10 hours postmortem. No leucocytes were found in the tissues. There was hemorrhage about the small vessels of the brain and thinning of the interradiary and tangential fibers in places. The writer concludes that a case not manic or melancholic but paranoiac or possibly confusional can show severe changes of a noninfectious character. This is the type of case we meet repeatedly in the literature, until we come to agree with Goldstein<sup>24</sup> (1910), that there are few uncomplicated cases in the literature; and we must finally agree that there can be no uncomplicated cases, if our methods of diagnosis and of pathological examination are adequate. Certain it is that the number of supposed uncomplicated cases has become remarkably few with rapid advances in accurate diagnosis and accurate protocol taking.

In 1906 Klippel and Lhermitte<sup>25</sup> described changes in the spinal cord of cases of dementia præcox. One case was that of a male of twenty-nine in which the cord showed changes in the posterior columns exactly like those seen in tabes. Another case, age thirty-five, gave changes in the lateral columns, without meningitis and without cord symptoms clinically. One of these writers<sup>26</sup> had previously (1904) described cases of dementia præcox in which there were no changes in the vascular connective tissue elements of the brain but only in the neuroepithelial elements. There were no leucocytes, no diapedesis, no hyperemia, no proliferation or degeneration of the walls of the vessels. He concluded from his studies that dementia præcox was a degenerative disease of the neuroepithelial elements of the brain and cord without involvement of the vascular connective tissue elements and that where the latter appeared to be involved, the involvement was due to intercurrent disease. In 1909 Moriyasu<sup>26</sup> presented nine cases, studied in extenso, in which he showed that the fibrils were fragmented and distinctly decreased in number. Changes in the ganglion cells were definite and distinct but, in accord with all workers, the changes in the ganglion cells were not characteristic of dementia præcox. Vascular changes were not significant. The glia nuclei were increased about the blood vessels, especially in the white matter and there was satellitosis about the pyramidal cells. He also says that changes in Clarke's column of cells in the spinal cord are constant. No plasma cells were found in any of his cases. He studied seven females and two males, ranging in age from twenty-five to fifty-three. The duration of the disease was from a few days in one case to two

years in one case. One died of erysipelas, two died suddenly, cause not given, and the cause of death was not given in the other six cases. To have quoted one thorough worker is to have quoted the rest most completely. Klippel and Lhermitte mention a pigment increase in the neuroglia (quoted by Moriyasu<sup>27</sup>). Mondio<sup>28</sup> (1905) says that the changes in the ganglion cells are those seen by others in idiocy and in the intoxications. Obregia<sup>29</sup> mentions chromatolysis and meningeal thickening with proliferation of the cells of the meninges and of the vascular adventitia.

Glia increase both cellular and fibrillar, with changes in the cells and fibrils; pigmentation of the nerve cells and of the glia and pigment lying free in the tissues or about the vessels or in the vessel walls or lumen; the presence of small lymphocytes or of leucocytes; loss of myelinated fibers; degeneration products such as fatty and protogonoid granules; satellitosis and neuronophagia; have all been observed and recorded.

What are the microscopic findings in the frankly delirious cases? the confusional cases? the toxic cases? Perhaps the first good description of that picture is the one given by Jahn<sup>2</sup> in 1877. The vessels showed fatty degeneration and nuclear heaps and pigmentation. There was extravasation of blood and overgrowth of glia. The lymph spaces contained fat and the cell protoplasm showed it also. Next Rezzonico<sup>5</sup> in 1884 showed dilatation of the finest vessels, with fatty degeneration of their walls and emboli made up of groups of micrococci. He noted in his writings that Briand had already, in 1882, found bacilli in the blood in three out of seven cases of acute delirium. What threw many cases out of the delirious group and into the dementia præcox group or what corresponded to it, then as today, was the absence of any notable fever; and albuminuria was only slight. How many times have we seen that ourselves in cases that went rapidly to a fatal termination!

Next, as might be expected, a specific bacillus was described for acute delirium (Bianchi and Piccinio,<sup>30</sup> 1895). But the histological changes found were the same as those found in dementia præcox: clouding of the pia, edema of the brain, adhesions of the meninges, capillary hemorrhages, extravasation of blood pigment, emigration of leucocytes, acute cellular degeneration (Cramer, quoted by Binswanger and Berger,<sup>31</sup> 1901); increase of glia and vascular nuclei (Popoff, quoted<sup>31</sup>), swelling and degeneration of the myelin sheath (Schukowsky, quoted<sup>31</sup>), satellitosis, chromatolysis (Weber,<sup>32</sup> 1904).

What were the general findings in the psychoses by Nissl and Alzheimer and those working at about their time, in and out of their laboratories? Alzheimer,<sup>33</sup> in 1906, remarks that with the same tinctorial methods which enabled us to differentiate general paralysis from senile dementia, etc., we are unable to show differences in the simple psychoses. Pathological changes are not lacking, he states, but who will number the cells or fibers lost? This seems to be his idea of the proper direction for future research in the psychoses. Fat occurs in epilepsy and in many other conditions besides the infections and dementia præcox. Protogonoid substances occur also in amaurotic idiocy (Alzheimer<sup>33</sup>). The neurofibrils, as studied by the Bielschowsky method (Schütz,<sup>34</sup> Jena, 1908), have lost their network arrangement in the cell bodies in dementia præcox, general paralysis, and secondary dementia. Glia proliferation has been observed

in general paralysis, senile dementia, alcoholics, uremic psychosis, and typhus delirium (Nissl, quoted by Alzheimer<sup>35</sup>). Yellow pigment in the cell bodies, first claimed by Campbell<sup>36</sup> to be pathognomonic of senile dementia, was found by Alzheimer<sup>37</sup> to exist also in epilepsy, arteriosclerotic degenerations and in general paralysis. Then Rosenthal<sup>38</sup> (1913), demonstrated that changes in the glia resembling the ameboid glia, could be produced experimentally by the injection into rabbits of guanidin, sodium oxalate, or of foreign serum into a sensitized animal. Granular degeneration and neuronophagia were also produced. This writer demonstrated, also, that the ameboid glia were certainly not postmortem appearances. A series of non-insane cases studied by Vogt<sup>39</sup> (1901) showed degeneration of cells, increase in neuroglia, and exudate in non-insane cases. Tuberculosis, according to him, shows chronic changes in the nerve cells generally, and may show increase in the glia.

With the case of pseudotumor cerebri (Rosenthal,<sup>22</sup> 1911), there appeared the acute Nissl reaction so often seen in the infectious processes, and also ameboid glia, satellitosis, protogonoid granules, and lipoid inclusions in the cells, just as we have seen in all the conditions presented in this paper.

The earliest and, perhaps, the best statement of the meaning of all this, is one written in 1898 by Juliusberger and Meyer.<sup>40</sup> They were of the opinion that the changes found were "evidence of abnormal life processes in the cell" and this simple statement can scarcely be excelled today. We can add just one thing to it, perhaps, and that is to state a little more clearly just what those abnormal life processes are and just a little as to how they may be effected. We have done this when we consider the reactions of the human organism, with its fats, carbohydrates and proteins, to the foreign substances which invade it, whether or not as bacterial protein or fat, and when we add to our considerations the susceptibility of the individual, especially whether or not he is hypersusceptible. We were all interested, I am sure, when we read in the *Journal*<sup>41</sup> that the typical histological picture found in the organs in tuberculosis, had been produced experimentally by the injection of the waxes from the bodies of tubercle bacilli into several different laboratory animals. The whole of pathology itself seems rapidly to be resolving into just such considerations and possibilities.

The conclusion from the above work that certain cases of dementia præcox may be of toxic or infectious origin is almost foregone. With this introduction, I wish to present four cases from the records of the Worcester State Hospital, in which the toxic factor was a large one but was not recognized until autopsy or until certain special tests were applied, namely, the spinal fluid and blood examination by microscopical, chemical, and serological analysis.

The first case was that of a female, W. S. H. No. 23119, age fifty-seven, diagnosis dementia præcox. Onset was at forty-four to forty-nine, variously estimated. She was first admitted to the McLean Hospital in 1903. She had been an efficient worker but gradually came to think that people were talking about her, later expressing the idea that she was boycotted and that people pointed her out on the street. Then she heard the telephone buzzing all night and said that someone kept a lawn-mower going another night. She was finally found on

the roof where she went to get rid of the voices. At the Hospital, her conduct was quiet and natural but she seemed a little depressed and preferred not to talk about her troubles. She was discharged but had to return because the scrub-

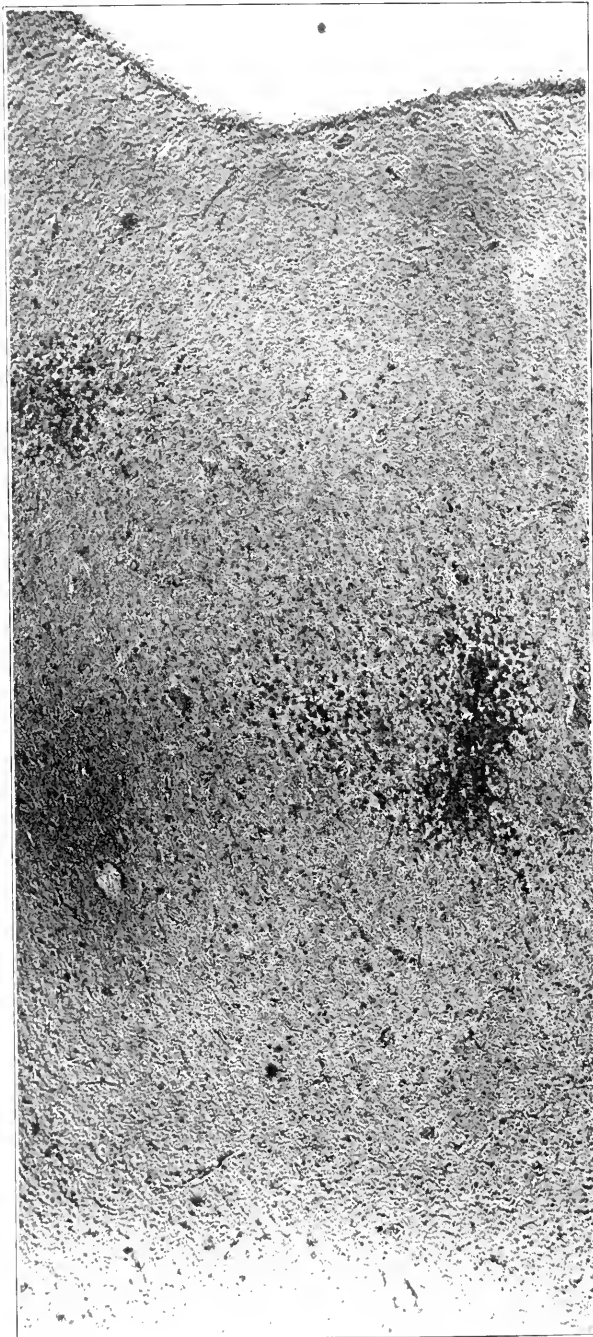


Fig. 1. Case 1, tubercular paranoid dementia praecox. Cortex of the right superior occipital gyrus, showing two massive processes probably related to the patient's tuberculosis. Section cut 50 micra. Scharlach-R stain. Photograph takes in cortex to white matter.

girl made faces at her, as did the priest in church, and voices dictated to her what she should do and derided her over the telephone. She became exalted and incoherent. She had some insight at first, saying that she could not understand how the voices could be heard, but insisting that she heard them. Physically her condition was good and she gained in weight about six pounds. She left the Hospital again in 1904 and returned in 1908. She was rather more demented than when she left, but physically she complained only of feeling tired all the time. Her weight dropped steadily from about 160 at last admission to about 90 pounds at death in 1915, a period of seven years. The postmortem examination showed adhesions in both pleural cavities, with fluid in the left cavity. Both lungs showed masses of tubercles, many of them coalescing and breaking down to form cavities. There were also irregular, circular ulcers in the ileum near the cecum. No discovery was made of the patient's condition clinically, when an x-ray examination of her chest at the outset would doubtless have revealed it.

The second case was that of a male, W. S. H. No. 26963, of unknown age but thought to be about fifty-two. He was admitted to the Hospital in 1910 and died suddenly in 1915 of coronary sclerosis with occlusion and acute hemorrhagic pancreatitis. He had led rather an irregular life, admitted having had private disease twice and being treated for gonorrhea at Tewksbury. Was told, a long time ago, that he had a chancre. He says he caught the fever at eighteen and his hair came out. He married twice; his second wife was a dissolute woman and was drunk most of the time. She left him about fourteen years ago. Patient admitted arrests and serving sentences and that he drank too much. The onset of his trouble was in 1909, coming gradually and finally terminating in his refusing to pay for a meal at a restaurant one day, on the ground that he owned the place. He was confused and rambling on admission. Said he could hear the voices of his parents and the voice of Jesus. Stated that "when he had his beard on" he could climb a telegraph pole and telephone to anyone. He became irritable, silly, demented. After three years, he said he could still see God and later that his father and mother still talked to him but that he was not bothered any more by it. His physical condition was always good. He took up the work assigned to him and had parole, sweeping up the yards, and so on. He had a scar on the prepuce, a palpable liver and a negative blood Wassermann. The spinal fluid examination was not made. The practice of not taking a Wassermann on the spinal fluid because the blood Wassermann is negative can not be too strongly deprecated, as this case with the scar on the prepuce and the abundant history and the next case show.

The third case was that of a male, W. S. H. No. 28853, age twenty-nine on admission, age thirty at death; total duration about two years. The patient was one of 26 pregnancies; 2 miscarried, 18 died in infancy, 1 died of convulsions, 2 boys and 4 girls still living and one of these has "fits." I cite these facts because there is some question in my mind as to whether the patient was not one of those congenital syphilitics whose disease becomes manifest at a later time than we are in the habit of thinking. (Dr. Abner Post, Neurosyphilis Conference, Grafton State Hospital, 1916.)

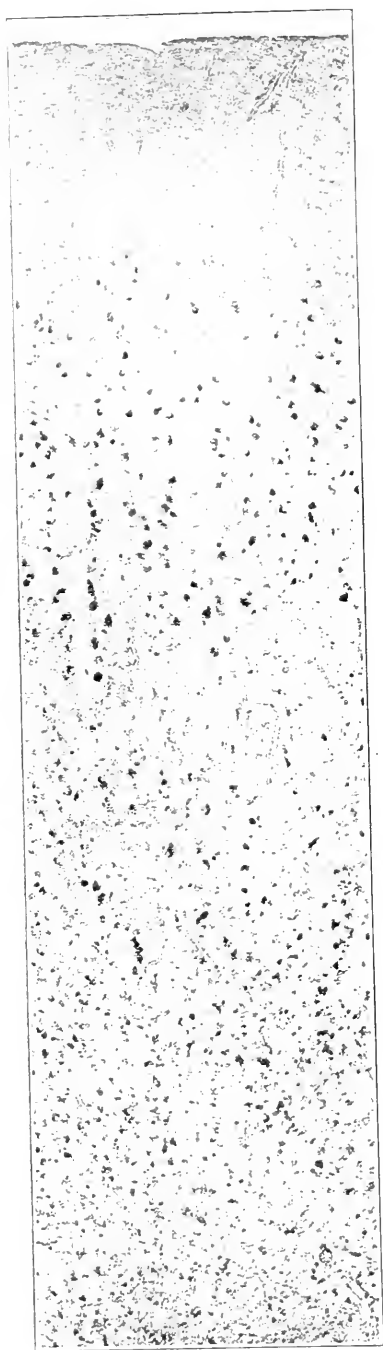


Fig. 2.

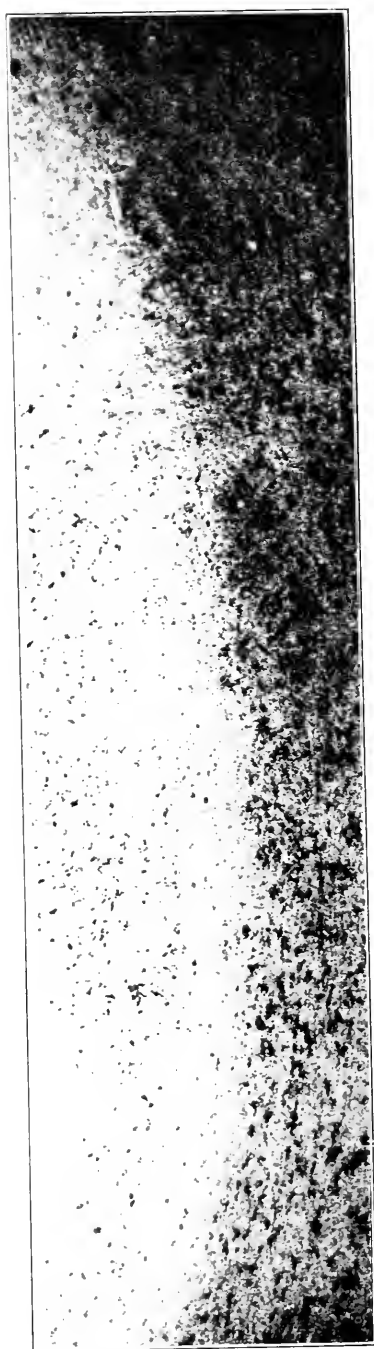


Fig. 3.

Fig. 2.—Case 2, alcoholic paranoid dementia præcox. Cortex of the left supramarginal gyrus, showing the fat to be chiefly in the outer layers of the cortex. Section cut, stained and photographed as in the previous case.

Fig. 3.—Case 3, parietic-like katatonic dementia præcox. Cortex and white matter of the left lingual gyrus, showing the large amount of fat in the white matter and the small amount in the cortex, with the sharp line of demarcation between the two which appeared in many sections. Staining and cutting same as before, but photograph includes only the white and the cortex at the bottom of a sulcus.

About 9 months before admission to the Psychopathic Hospital (No. 2632), the patient had become excessively alcoholic and oversexual. Two months before admission he had auditory hallucinations and thought he was about to be killed. He became maniacal, destructive to furniture and went out at night only partially clothed. He would remain from home a week at a time, contrary to his usual habits. On admission, he said he was "confused in his head." He was inaccurate about his recent movements and about the ordinary facts of his life and of his family. He was easily distracted and had exalted ideas about his talents as a singer, demonstrating until he was hoarse. Knee jerks were unequal and absent on the left. Admitted to the Worcester State Hospital, he was at first loquacious, showy, euphoric, but became irritable, sullen, resistive, mute, and had to be tube-fed. Later he had a convulsion, became wildly excited, then mute, untidy and stuporous. At the last he showed typical katatonic rigidity, with legs drawn up and massive, multiple decubitus developing. At the Psychopathic Hospital the provisional diagnosis had been manic depressive insanity, manic phase, then dementia præcox, and finally with the appearance of the Wassermann, general paralysis. The blood was positive. The spinal fluid showed 732 cells and the globulin and albumin were each 3 plus.

Autopsy showed a scar on the corona, there were dense adhesions in the pleural cavities and fresh tubercles or gummata were present in the lungs. The pia was thickened and showed whitish plaques. The brain weighed only 935 grams. The frontal poles were pointed, the right being much smaller than the left. The parietal regions were especially soft. The left cerebellar hemisphere was smaller than the right. The posterior columns of the spinal cord were almost confluent.

The fourth case is that of a negress, W. S. H. No. 30074, age nineteen, who was admitted to the Psychopathic Hospital (No. 5918, No. 6412), on March 20, 1916, transferred to the Worcester State Hospital March 29, and died April 2. On March 15 she began to fail and since she had failed to receive a certain letter from a certain young man, and since she was a mental case, the two facts seemed connected in some hidden manner. But on March 19 she had complained of malaise, anorexia, pain in the epigastrium and later of a "bursting headache." Her temples beat and her neck felt stiff. That evening, while setting the table, she disappeared and was not found until the next morning in an empty room next to her own, where she lay with her throat cut. She slowly recovered from the stupor in which she was found, when she explained that she had been drawing water when her head began to swim and she did not know anything more but that she had felt crazy. Her replies at the Hospital were inaudible, given only on close questioning, and she was stupid, apathetic, and indifferent. March 20 she developed cerea flexibilitas, muttered through her teeth, and retained her saliva. The breath had a foul odor. She said the doctor told her she was going to have a baby, but admits that she had a menstrual period only the week previously. Pulse was 132, temperature 100-101° F. constantly, respiration 20. She became restless, mute, untidy, resistive, at times shouting and at the end picking at the bedclothes. On March 30 there was a trace of albumin, numerous red blood cells, and numerous hyaline and granular casts in the urine. The

sputum was thin, green and purulent, and showed leucocytes and a bacillus morphologically like the influenza bacillus. The postmortem examination was practically negative and the case was reported as one of katatonic exhaustion or

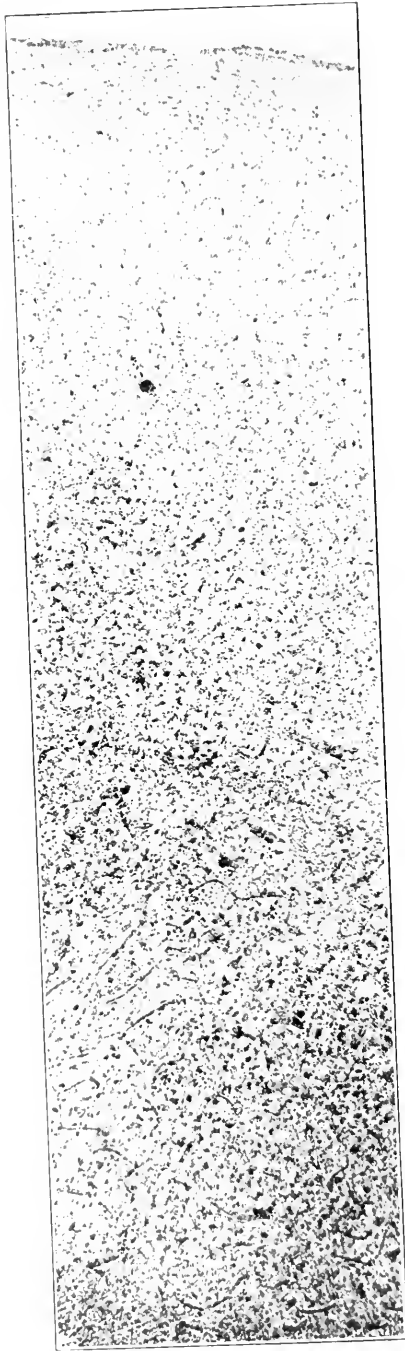


Fig. 4.--Case 4, influenza katatonic dementia præcox. Cortex of the right quadrate gyrus, showing the distribution of the fat in the small elements of the entire cortex, fairly evenly distributed. Staining, etc., as in Cases 1 and 2.



"Hirntod." But cultures taken from the heart's blood at autopsy showed a bacillus which, in its morphology and cultural characteristics, was the influenza bacillus.

Summarizing, we have two cases of the paranoid form of dementia præcox, both of rather long duration, one male and one female. One died of tuberculosis, the other of hemorrhagic pancreatitis and coronary sclerosis. And we have two cases of the katatonic form, both of comparatively short duration. One died of paresis, the other died of influenza. In one case of the four, only, could the mental diagnosis be questioned. The parietic case could be settled only with the Wassermann technic. The paranoid case with the alcoholic history and the history of chancre might be questioned. All but this one died of an infection. In two of these the infection undoubtedly ran parallel to the mental symptoms. In the third, which died of tuberculosis, is it too much to imagine that the mental symptoms also paralleled the physical disease?

The microscopic findings were studied by the photographic method on twenty-four sections from each side of the brain in each case. These were arranged according to what we know of the great functional subdivisions of the brain—the pre-Rolandic and the post-Rolandic, the latter being divided into parietal, temporal and occipital to correspond to general sensibility, hearing, and sight.

The stain used was the Scharlach-R for fat, because it is the most general stain we have for degeneration products of the nervous system and because it photographs so well.

The sections were cut at 50 micra with the idea that, if fat were found, it might present itself in some sort of radial or stratified way which would fail to show in thinner sections. This did not prove, in general, to be the case in this series but the method was carried out, as it appeared logical and gave fair photographs.

In general, the fat was found to be fairly evenly distributed over the cortex, with the exception of that in the alcoholic case. Here it was more marked in the general region of the second to fourth layers, or at least to the layers lying more externally. In the tuberculous case, there was a distinct massive process in several regions. In the parietic case, the fat was more abundant in the white matter, and this more in the post-Rolandic regions, corresponding to the areas that were noted at autopsy to be the softest. The parietic case was the only one showing any considerable fat in the white matter.

A hint has been given in the above at the topographic idea and at the stratigraphic idea. So far as I have been able to determine, only two writers have attempted any definite correlations between disease of cell layers and dementia præcox, or disease of brain areas and dementia præcox. Cotton,<sup>12</sup> as admitted by Alzheimer,<sup>13</sup> was the first to correlate disease of the second and third cortical layers with dementia præcox, mentioning at the same time that the fat occurs over the entire cortex. Southard<sup>14</sup> was the first to claim a relation between disease of the post-Rolandic areas and dementia præcox of the katatonic form and between disease of the frontal areas and the paranoid form. Later, Southard and his coworkers have shown some connection be-

tween lesions of the angular gyrus and katatonia,<sup>45</sup> gliosis of the thalamus and dementia præcox,<sup>46</sup> and cerebellar hemiatrophy and katatonia.<sup>18</sup>

Other writers have laid emphasis in their cases on a predominance of the pathological findings in one layer or another or in one brain area as against another, but no one has made any special claims for these observations before the writers mentioned above. Thus Alzheimer<sup>43</sup> mentioned changes in the deeper layers at first, later pointing out that they occurred in the more superficial layers to a greater extent. Klippel and Lhermitte<sup>47</sup> found the changes more in the zones of association, in the large pyramids of the third layer of cells and in the fusiform cells of the sixth layer of Hammarberg. Dunton,<sup>47</sup> in a case of katatonic dementia præcox dying of pneumonia, found chromatolysis most marked in the fifth layer of Hammarberg. Lubouchine<sup>48</sup> mentions the molecular layer and the layer of the small pyramids; Lannois and Paviot<sup>49</sup> mention the Purkinje cells of the cerebellum; Koller<sup>50</sup> mentions increase of glia in the superficial layers and in the white matter; Elmiger<sup>51</sup> mentions the free edge as being most affected; Orton<sup>52</sup> finds the ameboid changes more in the deeper layers and in the white matter; Sioli<sup>53</sup> finds more change in the upper layers; Mott<sup>54</sup> in the infragranule layers. As for the fibers: Cramer<sup>1</sup> mentions thinning of the interradiary and tangential systems; Weber,<sup>32</sup> the supraradiary network; Eisath-Hall,<sup>55</sup> Meynert's layers. All have support in the shape of corroborative work by other investigators.

Dunton<sup>47</sup> and Maschtschenko<sup>56</sup> claim that the frontal lobes are more affected; Zalplachita,<sup>57</sup> the frontal and central; Orton,<sup>52</sup> that the temporal and occipital are less affected than other parts; but no one has, so far as I can learn, investigated the question of topographical distribution in, and for itself, except the writer mentioned above.

The four cases here presented can add little of a decisive nature to these great questions. Only one case presents anything of interest to the strati-graphic question. That is the case of the paranoid with the alcoholic history, who died of hemorrhagic pancreatitis and his history and physical examination make the diagnosis of dementia præcox somewhat doubtful. However, in his case we do not have to consider the effects of an intercurrent or of a causative infection upon the cell picture, as we do in the three other cases. In his case the fatty changes were more in the outer layers. Topographically, his case was rather indefinite, the fat occurring mostly in the areas of audition and of general sensibility. The fact that the fatty degeneration is rather marked in the temporal regions in this case is especially interesting in view of the continued auditory hallucinations which the patient experienced, but the fact of its absence in other areas may mean either that the cells in those areas are exhausted or that they were not affected, at least at the time of the patient's death. To determine whether they were really exhausted would be an especially interesting research in view of ideas entertained that there is a relation between frontal lobe lesions and paranoid dementia præcox.

The idea that the power of the nerve cells to produce fat has been exhausted in those areas where the fat is not found, is given support by the findings in the second case of paranoid form, dying of tuberculosis. This case

was especially chronic, lasted longer than the first case, and clinically showed much more profound deterioration. From our knowledge of the occurrence of fat in the various pathological processes, we should expect to find an abundance of it here, and yet we find much less than in the first case. The idea gains credence that the fat-producing power is exhausted. In this case there were large masses of fat present, reaching macroscopic size in places, and doubtless connected with the tuberculous processes in the lungs and the intestines, but in general the whole cortex appeared exhausted.

The case of the young katatonic who died of influenza, has a most interesting distribution of the fat in the small elements of the cortex, especially in the glia cells and the blood vessel walls. The blood vessels are especially prominent, also, even the finest capillaries standing wide. The fat is distributed here in all layers and in all areas. It is in the most labile elements, the elements which are the most easily affected according to all writers, and the disease process was so fulminating that the nerve cells themselves have scarcely had time to react. Perhaps this case, together with the two previous ones, may give us some idea as to the fat-producing period of the nerve cell. It is evident that no topographic or stratigraphic ideas could be drawn from this case.

The last case, that of the paretic with katatonic mental picture, is striking by reason of the enormous amount of fat to be seen, and because the fat is confined for the most part to the white matter. The question arises here, as in the cases above, whether the cortex is involved at all or whether it is exhausted. Perhaps we have a type of paresis involving the fibers more especially and the gray matter rather less, just as it has been suggested (Southard<sup>28</sup>) that there are two types of feeble-mindedness, the type with plenty of pathways but little to go over them and the type with plenty of receivers and senders but few wires. The topographical significance in this case is great because the chief degeneration was found in the post-Rolandic regions, corresponding to the katatonic picture presented by the patient. And at autopsy, the especial softness of the parietal areas was noted.

With such an array of pathology as presented in the literature and in the histories and examinations of these patients, one should hesitate to make an unqualified diagnosis of dementia præcox, in the manner in which such diagnoses are made in many hospitals. There are few uncomplicated cases of dementia præcox. The question of a faulty diagnosis, the placing of a patient in the class of dementing psychoses of unknown cause, is often a difficult one to settle, but the attempt should always be made and methods should be added to the diagnostic facilities of most of our hospitals for the insane. Even in this small number of cases, the paretic one would most certainly have been missed had the patient's blood proved negative to the Wassermann examination and if it had been examined from certain of our hospitals. I have repeatedly taken the spinal fluid postmortem, where the examination was refused during the life of the patient on the ground that the blood was negative, and I have repeatedly found it positive to the Wassermann, chemical, and microscopical examination. In the case of the katatonic, the true picture would never have been revealed,

had it not been for the extensive bacteriological examination postmortem. Morphology is not enough to identify an organism. Extensive culturing, before and after death, and a microscopic examination of the tissues at autopsy, with the well known stains for bacteria, will often yield results in many cases of our so-called dementia præcox. Do not forget to stain for the tubercle bacillus. And soon we will come to a splitting of the dementia præcox group, here in America, into a lot of little groups, each on its own etiological basis. The beginning has already been made in splitting off those with a positive Wassermann reaction and other positive tests on the spinal fluid. Who knows how many are caused by influenza or tuberculosis or alcohol?

Beyond this talk of the individual case stands the interest in the group and for most of us, this interest crystallizes into the demand for the *why* of the mental picture. Like the why in any question concerned with the human body, we must go for the solution to physiology, before we can understand abnormal functionings. The brain is divided into several great regions according to this point of view, and hence our necessary interest in topography. On the mental side, in recent years, great efforts have been made to get at the roots of the ideas entertained by psychopathic subjects. Such efforts have rapidly departed from the teachings of the original founders and have drifted into a miserable, nondescript state which I have been tempted to call "neo-mysticism." Such a state was far from the minds of the founders, I am sure, for they said, in speaking of hysteria,<sup>59</sup> that the mechanisms which they had discovered could develop only on a constitution predisposed. And I take it that some of our ultra-moderns, even, are coming around that way, for I note that some account for their lack of success with the method by saying that the patient "did not have the 'stuff' in him," meaning by that, I suppose, that the patient was defective from the start. What makes the whole thing so mystical is not that its advocates deny the operation of physical elements, but that they behave as if they did. It is of little moment for one to admit that there is a constitution behind the patient's trouble, if in the next moment he tries to right the patient's difficulty by letting loose some pent-up ideas from the subconscious personality of the individual, such ideas always to be sexual in nature. This I take to be the gist of the difference between "neo-mysticism" on the one hand and Freudianism of the true sort with its psychoanalytic method, on the other. Some writers and workers have come to recognize this difference and so we see the attempt being made to resolve character into other elements than the sexual alone, and I think a truer picture will be presented—though we run the risk of being suspected of having certain complexes ourselves, you know, by trying thus to avoid the issue.

But what has come out of all this is a method, and the method can be of value to the anatomist. If this method can resolve the faulty character into its elements and these elements can be correlated with the abnormal functioning of known physiological regions, we have an extremely valuable method, indeed. Something of this sort might be expected from the introspective method of experimental psychology. By introspection, under experimental conditions, it is possible to resolve complex feelings, emotions, and ideas into simpler

elements which are visual, auditory, kinesthetic, etc., but we can never get patients to introspect under experimental conditions. Is not the psychoanalytic method the one to be used in resolving the patient's experiences into elements? Something may also be expected to come from the study of the behavior in the sense used by the experimental psychologist whose special field is the behavioristic side of physiology. But the study of the behavior of an insane individual is aided by the explanations of that individual, if those explanations can be analyzed and how can they be analyzed at the present time, except by the psychoanalytic method? Let us take, for example, the sign so often seen in dementia præcox, confusions of all sorts, and in the deliria—the removing of the clothing. The patient is said to be denudative. The reasons for the removal of the clothes under improper conditions, may be various. One may say it is a sign of exhibitionism, another that the clothes irritate the patient because the skin is hypersensitive, and so on almost *ad libitum*. But we may gain something, if we ask the patient about it. He may give a valuable answer directly or we may arrive at a valuable conclusion as to the reason by piecing his replies together, as the psychoanalytic method does.

Apropos of asking the patient questions, I am reminded of a striking incident which was observed during an intraventricular treatment for paresis. The patient became restless just as the needle was passing through the cerebral substance. Asked what the matter was, the patient replied that he would be all right, if the doctor would stop "making his mouth (patient's) fill up with spit"—and that his mouth was actually filling up with saliva was not imagination. Beside giving the reason for his restlessness, his reply gave a cue to a second very important point, that a cerebral injury may affect a gland-secretion. Ceni<sup>60</sup> has also shown changes in the cells of the testicles of roosters by subjecting them to cerebral injury.

The suggestion to use the psychoanalytic method along truly Freudian lines and not in the hazy way in which it is now being applied by his successors, for the most part, should be made now. But the application of the psychoanalytic method in this new way will have to wait until those using it recognize that there is something more beneath character than sex and that there is something more in personality than character alone. I mean by this that personality includes much more in its connotation than the word character includes. And only when these two are recognized, will the psychoanalytic method be of value for the topographical study of the cortex lesions of the insane and for the correlation of those topographically distributed lesions with the mental picture presented by the patient.

The requisites for future advance, then, are the recognition of unrecognized work: recognition of the fact that the central nervous system of dementia præcox patients gives evidence of abnormal life processes in the cells; that the same changes are present in the intoxications and the infections, in the confusions and the deliria; that there are methods at hand for diagnosis which are not being used; and that the diagnosis is often difficult and often wrong and will often be wrong when we have exhausted all our methods, but an intelligent attack will have been made.

## CONCLUSIONS.

1. That cases of dementia præcox, of confusional insanity, or of delirium, of pseudotumor, and of various other mental conditions have pathological changes which are similar.

2. That it is quite apropos to assume that certain cases of dementia præcox are due to infectious or toxic processes, in proof of which four cases are introduced, one of which is definitely syphilitic, one is alcoholic and possibly syphilitic, one is tubercular, and one is a case of influenza of fulminating type.

3. That cases which can be detected are being missed because of the lack of application of diagnostic methods. As an example, the refusal to permit a spinal puncture where the blood Wassermann is negative is especially decried.

4. That topographic studies are next in order, as offering a logical direction of research in solving the why of the mental picture—later to be followed by stratigraphic studies.

5. The idea is suggested that the psychoanalytic method may reduce the mental condition to elements which can be correlated with lesions topographically or stratigraphically disposed.

In conclusion, I wish to express my thanks to those who have shown their interest in this work, of whom there are many, and also to my Laboratory Assistant, Julius H. Stean, who assisted materially in making the sections and the photomicrographs.

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# ON THE POSSIBLE DERIVATION OF THE ACTIVE PRINCIPLE OF THE POSTERIOR LOBE OF THE PITUITARY BODY FROM THE TETHELIN PRODUCED BY THE ANTERIOR LOBE\*

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TWO views may be said to exist with regard to the place of formation of the active substance of the posterior lobe of the pituitary body: (a) that it is formed in the posterior lobe and may be a primary product of nervous tissue secretion; (b) that it is derived from the secretion of the anterior lobe which passes in part via the pars intermedia into the posterior lobe and so to the cerebrospinal fluid. Neither of these views has received sufficient experimental support to secure unequivocal adoption.

The former view is supported by a large amount of experimental work, the greater part of which shows that the active principle which on injection causes a marked rise in blood pressure, increase in the secretion of urine and milk, and contraction of smooth muscle, especially the uterus,<sup>1</sup> is found only in the posterior lobe while, as has been pointed out in the previous communication, neither extracts nor the pure substance tethelin, obtained from the anterior lobe, possesses these properties. To support the second view we possess merely indirect evidence. Of the two parts of the pituitary body the anterior apparently plays the more important role. Its secretion is necessary for the maintenance of life.<sup>2</sup> The work of Paulesco<sup>3</sup> confirmed by Crowe, Cushing and Homans<sup>4</sup> showed that extirpation of the whole pituitary body is fatal, this being entirely due to the loss of the anterior lobe since removal of the posterior lobe gave negative results. This may mean either that the secretion from the posterior lobe is not necessary for the maintenance of life or that conversion of a part of the secretion from the anterior lobe into posterior lobe substance can take place even after extirpation of the latter gland. Herring<sup>5</sup> points out that skates possess no posterior lobe.

Cow<sup>6</sup> found that in the cat definite channels of communication exist between the ventricular cavity in the infundibular stalk and spaces in the anterior lobe. In several extensive investigations, Herring<sup>7</sup> found that a uterine contracting substance can be obtained from the pars intermedia. He believes that the active principle of the posterior lobe is derived from cells of the pars intermedia. The epithelial cells of the pars intermedia secrete a colloid material which passes into the pars nervosa. The hyaline bodies break down liberating either a clear hyaline material or at times granules. Breaking down of the cells from pars intermedia presumably takes place in two stages; this would explain the formation of possibly two active substances one of which acts primarily on blood pressure and the other stimulates contraction of smooth muscle, especially the uterus. The presence of a colloid material in pars intermedia, also found in pars nervosa suggests a possible derivation of the active principle of the posterior lobe externally

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although Fenger<sup>5</sup> finds that the colloid masses secreted between the anterior and posterior lobes do not possess uterine contracting power.

Although several observers, among them Aldrich,<sup>9</sup> Engeland and Kutscher,<sup>10</sup> and Fühner<sup>11</sup> have reported the isolation of pure substances from the posterior lobe, no single pure substance of definite chemical nature has been isolated to which can be attributed the properties shown by posterior lobe extracts. This, of course, may be attributable to the presence of several physiologically active substances in the posterior lobe. The work of Dale and Laidlaw,<sup>12</sup> and Barger and Dale,<sup>13</sup> showing the specific action of  $\beta$ -iminözolyethylamine in producing contraction of the isolated uterus; the work of Ackermann,<sup>14</sup> Mellanby and Twort,<sup>15</sup> Windaus and Vogt,<sup>16</sup> and Pyman<sup>17</sup> showing the constitution of this substance and its relation to histidine; and the findings by Aldrich<sup>18</sup> of histidine-like substances in the posterior lobe; and more recently the isolation by Robertson of a definite chemical substance containing the iminözoly radical from the anterior lobe; these facts point very strongly to the supposition that the uterine contracting principle of the posterior lobe may be a resultant product from the splitting of the substance secreted by the anterior lobe.

Through the kindness of Professor T. Brailsford Robertson in supplying us with generous amounts of tethelin and giving us his unfailing interest, we were enabled to test this hypothesis and attempt if possible to split this substance, which on repeated tests proved to have no action in causing contraction of the isolated uterus of the nonpregnant cat or guinea pig, into products simulating posterior lobe extract in its action. Previous experiments with enzymes and bacteria gave negative results. We were then led to test the product resulting from the action of barium hydroxide on tethelin whereby the fatty acid radicle is split off, this reaction having previously been noted by Robertson.<sup>19</sup> In using the barium-split product we were well aware of the experiments of Guggenheim<sup>20</sup> who found on treating pituglandol with NaOH,  $\text{Ba}(\text{OH})_2$ , and PbO that inactivation of this substance had taken place, as well as those of Fühner<sup>11</sup> who used barium in the isolation of pure substances from the posterior lobe.

On addition of  $\text{Ba}(\text{OH})_2$  solution to a solution of tethelin an immediate flocculent precipitate is formed which quickly settles leaving the supernatant fluid perfectly clear but still possessing a straw-yellow color. Warming facilitates the flocculation. The excess of barium is precipitated with  $\text{CO}_2$  and filtered off. Soluble barium bicarbonate is removed by evaporation of the solution to a small volume; the barium is precipitated as the carbonate and filtered off. The filtrate is perfectly clear and possesses a light yellow color and retains the odor characteristic of tethelin solutions. Tests indicated no barium. The entire amino-nitrogen of the tethelin is found in this solution.

Experiments with the solution of tethelin-split product on the isolated uterus of virgin guinea pigs suspended in Locke and Ringer solutions were carried out in essentially the manner described by Dale and Laidlaw.<sup>21</sup> Difficulties attributable to hypersensitive uteri as noted by these authors were likewise experienced by us. Such uteri were rejected. Addition of tethelin-split product in doses given on the chart uniformly caused contraction; this could be repeated with the same muscle after washing in Locke solution and allowing it to come to rest. That

this action was not attributable to the action of tethelin or contamination by posterior lobe substance was repeatedly shown in control experiments. Neither could this effect be attributable to traces of barium in the solution since control experiments with solutions containing small amounts of barium also gave negative results. All experiments in which no uterine contraction was obtained on addition of the test substance were controlled by addition of either posterior lobe extract or ergamine which resulted in the usual contractions. A number of tethelin-split product preparations were made at different times and in all cases contraction was noted.

Experiments were likewise made to study the effect of the split product on the blood pressure of rabbits under ether anesthesia. As seen from Fig. 2 a slight but perceptible rise of blood pressure follows the injection of this substance. However, no preliminary fall is noted. With very dilute pituitary extract solutions small rises of blood pressure without a preliminary fall were observed and the effect could be repeated several times. Administration of larger doses of the same extract gave a preliminary fall followed by a rise.

While our experiments apparently indicate that we have split tethelin into products among which is a substance resembling in its action the active substance secreted by the posterior lobe, we are by no means certain that our method is the best for accomplishing this reaction or for obtaining a pure product. Since the nitrogen content of tethelin is low (2.58%) and that of  $\beta$ -iminozolyethylamine high (38%), the derivation of this latter substance from the former can yield only a small proportion. The delicacy of the uterine test as a means of detecting small amounts of this product is apparent.

We believe from the evidence presented that the active substance of the posterior lobe of the pituitary body is largely, or in part, derived from a splitting of the substance which is a product of the secretion of the anterior lobe and which contains an iminozoly radical.

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# STUDIES ON THE PROPERTIES AND ACTION OF TETHELIN\*

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THE results of numerous studies by various workers indicated the importance of the anterior lobe of the pituitary body as a growth factor, and showed that extracts of this lobe are relatively inactive physiologically, as contrasted with the extreme activity of extracts of the posterior lobe. However, it was not until the isolation of a definite chemical substance, tethelin, by Robertson<sup>1</sup> that the properties and action of the growth-controlling principle of the anterior lobe of the pituitary body could be more fully studied.

Tethelin is described by Robertson as a cream-colored substance which is readily obtained in powdered form. It is extremely hygroscopic and for that reason is best kept in vacuum tubes. The substance is soluble in water, ethyl alcohol, ether, chloroform and carbon tetrachloride, but insoluble in a mixture of one part by volume of absolute alcohol and one and one-half parts of dry ether. Its content of phosphorus is 1.14 per cent, nitrogen 2.58 per cent, of which one-half or 1.24 per cent is in the amino form, this being increased on complete hydrolysis (conversion of imino to an amino group) to 1.83 per cent or three-fourths of the total nitrogen. The presence of an unsaturated fatty acid is indicated by a saponification value of 87 milligrams of KOH per gram of substance and an iodine value of 33.2 per cent. Tethelin contains an iminazolyl radicle which indicates that possibly the physiologically active substance of the posterior lobe may be derived from it. On complete hydrolysis with barium hydroxide, followed by hydrolysis with dilute sulphuric acid, one of the decomposition products obtained is inosite.

Physiological experiments conducted by Robertson show that certain of the characteristic reactions produced by extracts of the posterior lobe—rise of blood pressure and pronounced diuresis—are not brought about by tethelin. On the contrary, intravenous injection produces a slight and transient fall in blood pressure and a slight increase in the amplitude of the heart beat. These results agree with the experiments of Hamburger,<sup>2</sup> Lewis, Miller and Mathews,<sup>3</sup> Herring,<sup>4</sup> Howell,<sup>5</sup> and others working with anterior lobe extracts.

Robertson<sup>6</sup> also carried out extensive investigations to determine the effect of tethelin on the growth of white mice. He found that administration of tethelin produces a marked retardation of the first portion of the third growth cycle, followed by acceleration of the latter portion of the same cycle. These results are in agreement with the observations of Aldrich,<sup>7</sup> Schäfer,<sup>8</sup> Wulzen,<sup>9</sup> and Robertson<sup>10</sup> on feeding anterior lobe to experimental animals. Cholesterol<sup>11</sup> was found to have a like effect on the growth of mice, although larger quantities are required. Tethelin given hypodermically to rats was found by Robertson and Burnett<sup>12</sup> to increase the rate of growth of the Flexner-Jobling carcinoma, and,

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likewise, to have the tendency to form metastases. In these respects the effects produced by tethelin were similar to those produced by anterior lobe.<sup>13</sup> Cholesterol<sup>14</sup> also produces a like effect. The action of tethelin, as well as other lipoids, on growth are those of a catalyser though the mechanism whereby such catalysers act is not known. Robertson<sup>15</sup> has also investigated the action of tethelin as an accelerator of tissue repair and found that it has a marked stimulating action on the healing of wounds.

On account of the importance of tethelin, as shown by the work of investigators previously cited, and its possible application as a therapeutic agent, it was considered of interest to study the properties and action of this substance further, particularly along the lines indicated by the subsequent headings. Reference to this work has already been made by Robertson.<sup>16</sup> The tethelin used for these

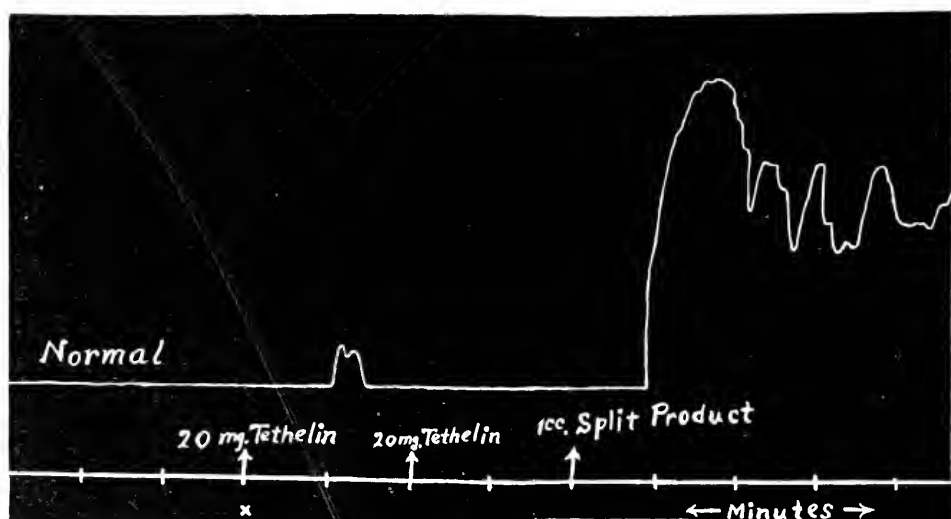


Fig. 1.—Showing effect of tethelin and tethelin-split product on the isolated guinea-pig uterus. 1 c.c. split product  $\equiv$  to 20 mg. tethelin.

experiments was generously supplied to me by Professor Robertson to whom I wish to express my thanks.

#### ANTIGENIC PROPERTIES.

Normal rabbits Nos. 843 and 850 were given six intravenous injections of 50 mg. each of tethelin dissolved in salt solution over a period of two weeks and bled ten days after the last injection. Alexin-fixation experiments, using one quarter of the minimum inhibiting dose of antigen and doses of rabbit serum of 1-10 cubic centimeters, were carried out in the usual manner. No fixation was obtained.

Four guinea pigs were given doses of tethelin, varying from 5 to 50 mg., intraperitoneally, and three weeks later each was given a second dose of 50 mg. Observations of the body temperature were made in a similar manner to the method used by Wells and Osborne<sup>17</sup> in their studies on the biological reactions of proteins. Only slight changes in body temperature—not greater than observed

# Effect of Tethelin Split Product on Blood Pressure of a Rabbit

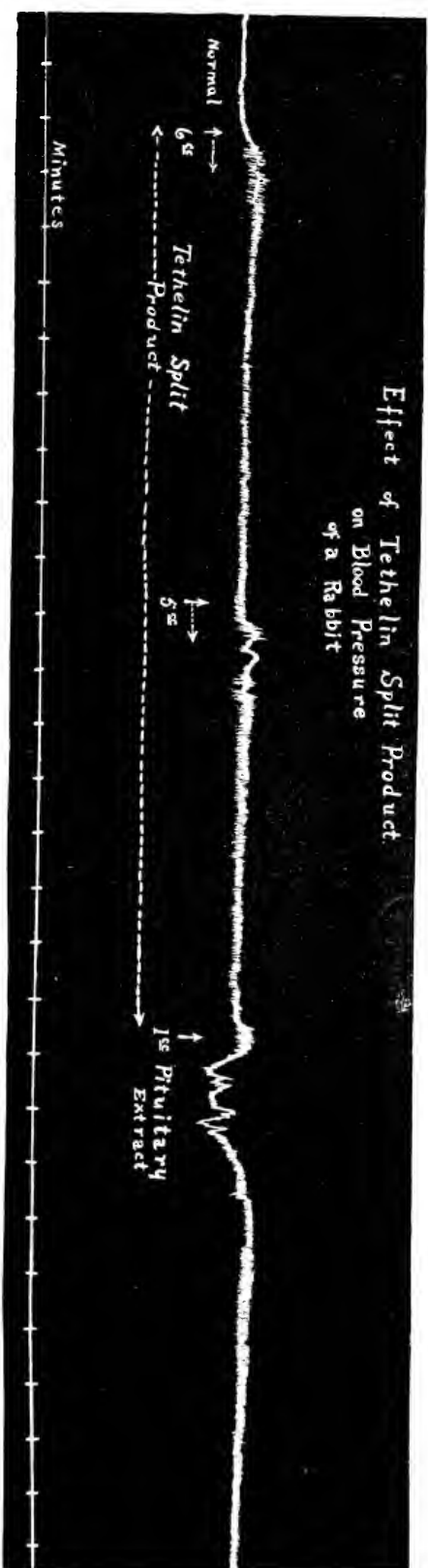


Fig. 2.—Showing effect of tethelin-split product and posterior lobe extract on the blood pressure of a rabbit under ether. 1 c.c. split product  $\text{---}$  to 20 mg. tethelin.

on injection of a similar dosage into a normal animal—were noted. The substance is likewise nontoxic; 100 milligram doses can be given intraperitoneally to a guinea pig without the animal's showing toxic symptoms.

These experiments, showing that tethelin is nonantigenic, are in agreement with the majority of the work on lipoids cited by Landsteiner<sup>18</sup> and the more recent work of Fitzgerald and Leathes<sup>19</sup> and Thiele and Embleton.<sup>20</sup> Adrenalin likewise possesses no antigenic properties, as shown by the work of Elliott and Durham.<sup>21</sup> The characteristic febrile response noted by Crowe, Cushing and Homans<sup>22</sup> on injecting boiled anterior lobe emulsions in states of experimental hypopituitarism can not be attributed to anaphylactic shock (this possibility was pointed out by them) produced by injections of the active principle of the anterior lobe.

The work of various observers, showing that the antigen used in the Wassermann test for syphilis can be wholly or in part replaced by artificial antigens,<sup>23</sup> such as cholesterol, lecithin, fatty acids, etc., led to the attempt to substitute tethelin for the usual antigen used in the test. Through the courtesy of Miss Grace A. Macmillan of the State Hygienic Laboratory, I was supplied with a number of sera and spinal fluids, which had previously been found by her to be triple positive. These tests carried out with the usual technic, using one quarter of the minimum inhibiting dose of tethelin as antigen and 1, 10 c.c. of the luetic sera, showed no inhibition of hemolysis. Tethelin is, therefore, incapable of acting as the antigen in the Wassermann test.

#### EFFECT ON BLOOD PROTEINS.

The effect of the injection of various substances, such as proteins, bacterial toxins, bacterial vaccines and living virulent bacteria, on the proteins of the blood

TABLE I.

EFFECT OF INJECTING 100 MG. TETHELIN INTRAVENOUSLY ON THE PROTEINS OF THE BLOOD SERUM OF RABBIT NO. 845 ♀. INITIAL WEIGHT 3 KILOS. ANIMAL LOST 60 GRAMS DURING EXPERIMENT.

Day of Experiment	Leucocyte count	Non-Protein %	Albumin %	Globulin %	Total Protein %	Albumin calculated as % total Protein	Globulin calculated as % total Protein	Protein Quotient
1	10700	1.3	5.2	1.5	6.7	78	22	3.5
2	.....	..	..	..	..	..	..	..
3	8800	1.3	4.4	1.6	6.0	73	27	2.7
4	11400	1.3	5.0	1.4	6.4	78	22	3.5
5	11800	1.3	4.6	1.6	6.2	74	26	2.8
6	11300	1.3	4.6	1.8	6.4	72	28	2.6
Averages		1.3	4.8	1.6	6.3	75	25	3.0
6 100 mg. tethelin intravenously at 10 A.M.								
6-4 P.M.	12800	1.4	4.7	1.5	6.2	76	24	3.2
7	10700	1.3	4.6	1.6	6.2	74	26	2.9
8	8400	1.2	4.5	1.8	6.3	71	29	2.4
9	.....	..	..	..	..	..	..	..
10	10800	1.3	5.2	1.4	6.6	79	21	3.8
11	8000	1.4	4.3	1.9	6.2	69	31	2.2
12	8600	1.3	4.4	2.0	6.4	69	31	2.2
Averages		1.3	4.6	1.7	6.3	73	27	2.8

serum has been the subject for extensive investigations in recent time by Rowe,<sup>24</sup> Righetti,<sup>25</sup> Hurwitz and Meyer,<sup>26</sup> and Schmidt.<sup>27</sup> The effect of tethelin on serum proteins has also been investigated, the determinations being carried out according to the micro-refractometric method of Robertson.<sup>28</sup> As seen from the following results, the injection of tethelin is without effect (Table I, II and III).

TABLE II.

EFFECT OF INJECTING 100 MG. TETHELIN INTRAPERITONEALLY ON THE PROTEINS OF THE BLOOD  
SERUM OF RABBIT NO. 846 ♀. INITIAL WEIGHT 2380 GRAMS. ANIMAL GAINED 60  
GRAMS DURING EXPERIMENT.

Day of Experiment	Leucocyte count	Non-Protein %	Albumin %	Globulin %	Total Protein %	Albumin calculated as % total Protein	Globulin calculated as % total Protein	Protein Quotient
1	11600	1.3	3.8	1.8	5.6	68	32	2.1
2	.....	..	..	..	..	..	..	..
3	14300	1.3	3.7	2.2	5.9	63	37	1.7
4	11800	1.3	4.4	1.8	6.2	71	29	2.4
5	12400	1.3	4.0	2.1	6.1	66	34	1.9
6	11700	1.3	4.4	1.6	6.0	73	27	2.7
Averages		1.3	4.1	1.9	6.0	68	32	2.2
6-10 A.M.	100 mg. tethelin intraperitoneally.							
6-4 P.M.	10600	1.2	4.5	1.4	5.9	76	24	3.2
7	11100	1.3	3.6	2.1	5.7	63	37	1.7
8	11300	1.2	3.9	2.1	6.0	65	35	1.9
9	.....	..	..	..	..	..	..	..
10	10600	1.3	4.8	1.3	6.1	79	21	3.8
11	9800	1.4	3.9	2.4	6.3	62	38	1.6
12	11800	1.3	4.2	1.8	6.0	70	30	2.3
Averages		1.3	4.1	1.9	6.0	69	31	2.2

TABLE III.

EFFECT OF INJECTING 100 MG. TETHELIN SUBCUTANEOUSLY ON THE PROTEINS OF THE BLOOD  
SERUM OF RABBIT NO. 847 ♀. INITIAL WEIGHT 2470 GRAMS. ANIMAL GAINED 200  
GRAMS DURING EXPERIMENT.

Day of Experiment	Leucocyte count	Non-Protein %	Albumin %	Globulin %	Total Protein %	Albumin calculated as % total Protein	Globulin calculated as % total Protein	Protein Quotient
1	8800	1.3	4.2	1.6	5.8	72	28	2.6
2	.....	..	..	..	..	..	..	..
3	7200	1.3	3.5	2.3	5.8	60	40	1.5
4	7600	1.3	4.0	1.8	5.8	69	31	2.2
5	8300	1.3	3.4	2.2	5.6	61	39	1.6
6	9400	1.2	4.5	1.5	6.0	75	25	3.0
Averages		1.3	3.9	1.9	5.8	67	33	2.0
6-10 A.M.	100 mg. tethelin subcutaneously.							
6-4 P.M.	10100	1.3	4.2	1.2	5.4	78	22	3.5
7	8000	1.3	3.9	1.7	5.6	70	30	2.3
8	7200	1.3	3.2	2.2	5.4	59	41	1.4
9	.....	..	..	..	..	..	..	..
10	7700	1.3	4.2	1.5	5.7	74	26	2.8
11	7200	1.3	3.5	2.2	5.7	61	39	1.6
12	7500	1.3	4.2	1.7	5.9	71	29	2.5
Averages		1.3	3.9	1.8	5.6	69	31	2.2

## EFFECT ON BLOOD COUNT.

The function of tethelin as a stimulator of the growth of granulation tissue in wounds is that of a catalyser of growth. It seemed possible that the action might consist in the production of an increased leucocytosis. That tethelin has no striking effect on the production of an increased leucocytosis is shown by the following experiments (Table IV).

TABLE IV.

EFFECT OF THE INTRAVENOUS INJECTION OF 100 MG. TETHELIN ON THE LEUCOCYTE COUNT OF RABBIT T. ♂.

TIME.	LEUCOCYTE COUNT.
9:00 A.M.	12300
9:30 "	100 mgs. tethelin intravenously.
10:00 "	11300
10:30 "	9600
11:05 "	10100
11:30 "	14700
12:08 P.M.	16200
12:40 "	11800
1:10 "	12600
1:43 "	11700
2:10 "	10000
2:40 "	10400
3:10 "	12200
3:45 "	10200
4:20 "	9700
5:20 "	12700

The effect of tethelin on the leucocyte count of rabbits under operative conditions was likewise determined. Two rabbits of practically the same weights were operated on for me by Dr. E. S. May. A four inch skin incision on the abdomen of the animal was made under nitrous oxide anesthesia. Rabbit No. 848 received 100 mg. of tethelin subcutaneously just after the operation, while No. 849 received none. The results are shown in Table V.

TABLE V.

TIME.	LEUCOCYTE COUNT.
Rabbit No. 848.	
Previous day	9:00 A.M. 8000
Day of operation	9:30 " 8100
"	10:30 " Operated—100 mg. tethelin
"	2:30 P.M. 7100
"	5:40 " 7200
Following day	10:00 A.M. 6700
Rabbit No. 849.	
Previous day	9:00 A.M. 10,200
Day of operation	9:30 " 13,600
"	10:15 " Operated—no tethelin
"	2:30 P.M. 16,200
"	5:40 " 12,800
Following day	10:00 A.M. 9,900



The effect of a 200 milligram dose of tethelin administered subcutaneously on the leucocyte count of human subject T.B.R. was also studied with the following results (Table VI).

TABLE VI.

TIME.		LEUCOCYTE COUNT.
Day previous to injection	10:30 A.M.	8400
"	6:00 P.M.	7700
Day of experiment	11:30 A.M.	8100
"	11:45 "	200 mg. tethelin subcutaneously Ring of hyperemia was noted, which disappeared shortly afterwards
"	2:00 P.M.	
"	3:40 "	8500
"	6:15 "	8900
Following day	11:00 A.M.	7200

No temperature change or symptoms were shown following the injection of tethelin. Differential counts likewise showed no appreciable change from the normal. That the slight increase in the leucocyte count following the intravenous injection of 100 mg. of tethelin shown by rabbit T $\delta$  can not be attributed to a specific effect of tethelin is shown by the work of Hamburger and Reufs<sup>29</sup> and Aschenheim,<sup>30</sup> who obtained similar increases of leucocyte counts on injection of proteins, foreign sera, and other substances.

#### GROWTH OF BACTERIA.

Certain bacteria can grow on a medium containing one per cent tethelin and a trace of salt. A number of generations of *B. coli* and *B. proteus* were grown on such media for a number of generations without the production of gas or indol. *B. bulgaricus*, however, does not grow on this medium. Gas was produced by *B. coli* on a medium containing one per cent tethelin and one per cent glucose. Evidently these organisms can derive sufficient nitrogen for growth by breaking down of the complex organic substance.

#### ACTION OF FERMENTS.

Attempts to split tethelin by the action of ferments gave negative results. Grüber's trypsin, shown to be active by parallel experiments on casein, failed to produce an increase of amino nitrogen after twenty-four hours action on tethelin at incubator temperature. Evidently the complex structure of tethelin makes it resistant to the action of trypsin. In the case of pepsin it has been observed by Dale<sup>31</sup> that whereas pepsin failed to reduce the pressor or diuretic principle, trypsin reduced the action on blood pressure and urinary flow to nil after a few hours digestion. An active lipase obtained from a pig's liver likewise failed to produce a splitting of tethelin, as judged by increase of titratable fatty acid. Mueller<sup>32</sup> found that cholesterol esters resist saponification by the ordinary lipases, which easily hydrolyze neutral fat. A comparable resistance is evidently shown by tethelin.

That the active principle associated with the posterior lobe of the pituitary body is entirely absent from the anterior lobe was found by a number of observers, previously cited, who have shown that this is true in regard to inability to

produce a rise of blood pressure and diuresis. It has also been shown by Herring<sup>33</sup> that extracts from the anterior lobe do not stimulate the uterus to contract. This has been confirmed with respect to tethelin by a number of experiments on isolated cat and guinea pig uteri. The well known method used by Dale and Laidlaw<sup>34</sup> for the standardization of pituitary extracts was employed. The uteri were suspended in 300 c.c. of either Ringer's or Locke's solution, and amounts of tethelin varying from 10 to 100 mg. added. No contraction was observed. Addition of either pituitary extract or ergamine produced the usual contraction in every instance. This indicates that tethelin possesses none of the characteristic properties of pituitary extracts as well as freedom from contamination by posterior lobe substance. Tethelin which had been subjected to the action of trypsin and lipase likewise failed to stimulate uteri to contract.

**SUMMARY.**—The properties and action of tethelin, the active principle of the anterior lobe of the pituitary body, have been studied. It was found that tethelin is nonantigenic and nontoxic; hence its use therapeutically is warranted. It can not be used as the antigen in the Wassermann test, has no effect on the proteins of blood serum, does not cause a specific hyperleucocytosis, can be used as a medium for the growth of certain bacteria, is not split by trypsin or lipase, and does not stimulate contraction of the isolated cat or guinea pig uterus.

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# MEMORANDUM AS TO THE MEANS OF PREVENTING AND TREATING TYPHOID FEVER, PARTICULARLY IN ARMIES, UNDER THE CONDITION OF WAR\*

BY FREDERICK P. GAY, M.D., BERKELEY, CALIF.

PROPHYLACTIC vaccination against typhoid fever is now acceptably the most important weapon for the prevention and eventual abolition of this disease. Adoption of similar methods to the paratyphoid fevers will unquestionably lead to their disappearance as well. The following statements are presented as a personal impression of how the important facts we already possess concerning vaccination against typhoid may best be applied, and also further suggestions as to lines of investigation that may lead to further advances.

## A. PROCEDURES IN ANTITYPHOID VACCINATION WHICH MAY BE REGARDED AS ALREADY SUFFICIENTLY PROVEN TO BE APPLIED.

1. In times of peace and of war armies should be completely immunized against typhoid and paratyphoid fevers by means of vaccination. Every effort should be made, moreover, to extend opportunities for vaccination to the general population, where prophylactic results hitherto have not been so thoroughly good as in armies, owing, at least in some part, to the fact that entire groups of individuals have not been vaccinated. We regard the most successful results of vaccination in the United States Army as due somewhat to the fact that one hundred per cent of the men have been vaccinated. If the entire population became vaccinated, there is reason to anticipate that typhoid fever would eventually disappear.

2. The type of vaccine to be employed in immunization should be uniform.

It should be prepared either in a large central laboratory for distribution over the entire country, or might be prepared in several laboratories in different parts of the country under rigid directions and then controlled by a central laboratory. Numerous varieties of typhoid vaccine have been prepared and advocated. It is difficult or impossible to estimate which of these vaccines represents the best preventive against typhoid fever, but that there is a difference in the protective value of one vaccine over another is undoubted. Certain criteria may, however, we believe, be accepted as essential in the preparation and administration of vaccines. The important points in the preparation of the vaccine itself are, we believe, the following:

(a) A typhoid vaccine to be most efficient prophylactically should be polyvalent, containing representative strains of the typhoid bacilli which have been shown to vary, and particularly containing freshly isolated strains in preponderance rather than old laboratory strains of the organism.

(b) The method of killing vaccine may be either by heating to a moderate degree, not above 55° C., or by the admixture of alcohol or ether. I am personally inclined to believe that one of the latter methods is the better.

\*Submitted to the National Research Council by Frederick P. Gay, as Member of the Committee on Medicine and Hygiene.

(c) Any vaccine employed for army administration, particularly in times of war, should contain equal parts of typhoid (polyvalent) and of paratyphoid alpha and paratyphoid beta, which should also be compounded of a number of strains. It is probably not necessary to include the paratyphoid vaccines in the immunization of civil communities.

### 3. Mode of inoculation of vaccine, including dosage.

In addition to the usual procedures of asepsis and choice as to the time of day for administration, care in reference to diet, observation as to the few possible contraindications, a uniform procedure of administering the vaccine should be adopted which should include the following:

(a) The total immunizing treatment should include not less than 3,000 million of each of the organisms, typhoid and the two paratyphoids, employed, which should be administered in equal parts simultaneously. Recent results in Europe lead us to believe that the three vaccines may be given in doses as large as 1,000 million each without any severer results than those obtained by the use of a single organism. Under these conditions three injections would suffice to complete the treatment, but if the reaction is too severe, the dosage could be diminished and a larger number of injections be given, the total amount of bacteria employed being more important than the number of inoculations.

(b) The injections should be given on alternate days, a method which would hasten the time of protection, facilitate the completion of inoculations of large numbers, and will apparently produce as lasting immunity as vaccinations at longer intervals.

### 4. Methods should be taken to test the lack of immunity in vaccinated individuals.

We believe that the typhoidin test offers an indication of failure of the individual to respond properly to vaccination when the test is negative and thereby serves as an indication for revaccination. The test should not be employed as a means of insurance of absolute protection against typhoid fever, which could probably never be assured. We suggest, therefore, that every vaccinated individual be tested one month after completing treatment by the typhoidin test, which, if negative, indicates need of further injections. Each individual should further be tested at yearly intervals in a similar manner.

## B. SUGGESTED LINES OF INVESTIGATION IN ANTITYPHOID IMMUNIZATION.

### 1. Tests of the comparative protective value of sensitized vaccines.

We believe that sensitized vaccines give indications of protecting better against typhoid fever than unsensitized vaccines. We suggest, then, that it would be thoroughly justifiable and of scientific value to vaccinate in an army certain parts of units with sensitized instead of with unsensitized vaccine in the same doses, so as to test under the subsequent conditions of like exposure the relative protective value of the two methods. This procedure could not be recommended if there were not sufficient evidence to lead us to believe that sensitized vaccines are at least as protective as unsensitized.

### 2. If similar groups of men are vaccinated by two or more methods, a subsequent study of the presence of antibodies in their blood and of typhoidin re-

actions would be of great value as correlated with their actual protection against typhoid fever.

3. It has been asserted that the typhoidin test is not absolutely specific in that typhoid vaccinated individuals may give a positive reaction with a similar preparation of paratyphoidin alpha. We regard this fact, if true, as in no way militating against the use of the reaction as indicating revaccination. It is important, however, to test out how far typhoid vaccinated individuals will respond to extractives, not only of paratyphoid but of other bacteria, and the similar behavior of individuals that have been vaccinated against paratyphoid as well as typhoid organisms. (An investigation of this problem is already under way in this laboratory.)

#### C. TREATMENT OF TYPHOID FEVER.

A few cases of typhoid fever will unquestionably still occur in spite of the best methods of prophylactic immunization that can at present be devised. These cases have been present even in the relatively successful results that have attended vaccination in the United States army, and have increased during the recent conditions of greater exposure on the Mexican border. In actual warfare they would be still further increased, as is evidenced by the 1500 or more cases that have occurred in the British army during the first two years of the war. It is important that these cases should be segregated as soon as diagnosis is made, and treated. We regard the recent results with the intravenous injections of vaccine, particularly of sensitized vaccine, as not only promising but as indicated in all cases of typhoid fever, as shortening in the majority of cases and as aborting in about one-third of the cases the course of the disease.

#### D. TYPHOID CARRIERS.

Every method should be utilized for the detection of typhoid carriers in armies, and further studies should be undertaken as a possible means of cure of these conditions, which have proved so refractory to the present time. We regard the present investigations which are being undertaken in several places on the carrier condition in rabbits as most promising in this direction.

# THE PREPARATION OF THE PURE SODIUM PHOSPHITE AS AN ANTIDOTE FOR MERCURIC CHLORIDE POISONING\*

BY G. A. LINHART, BERKELEY, CALIF.

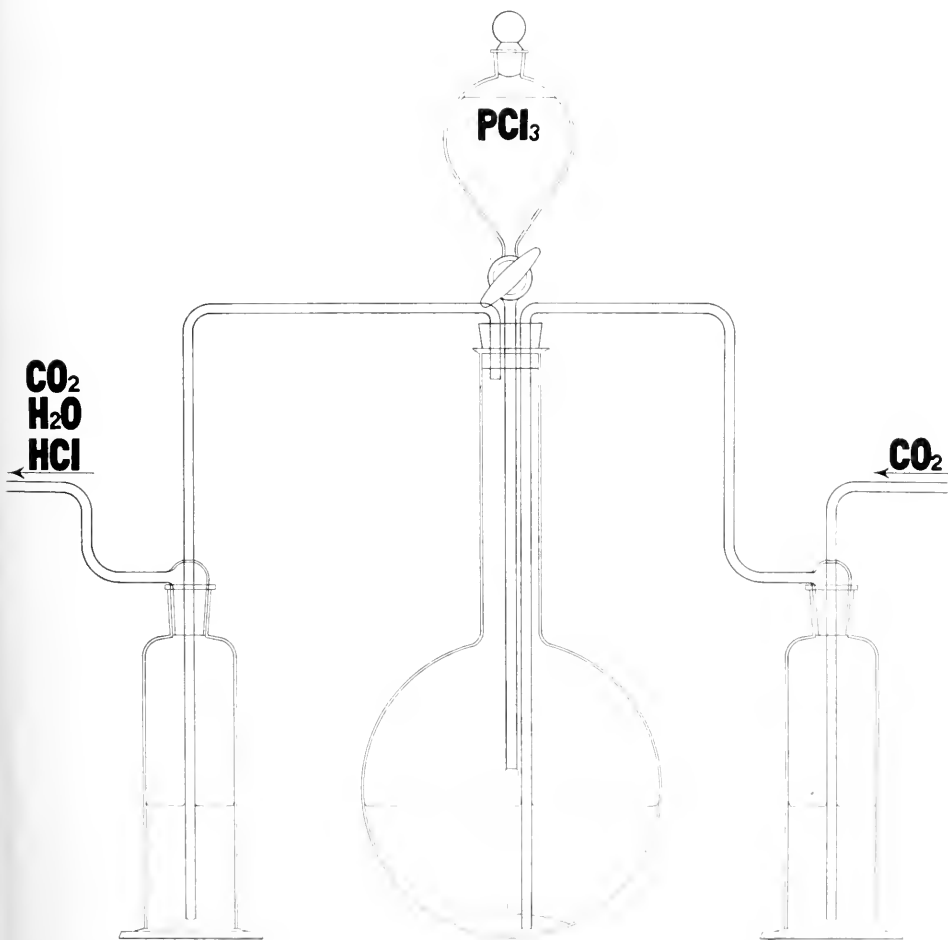
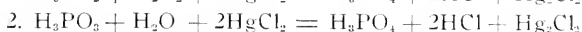
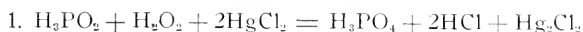
IN June, 1913, a preliminary paper was published by the writer,<sup>1</sup> dealing with the transformation of mercuric chloride to calomel in the body in cases of mercuric poisoning. Unfortunately, the work had to be discontinued owing to lack of money for the purchase of materials and animals. However, it is very gratifying to find that several investigators soon took up the problem, and, after extensive investigations, have conclusively substantiated my results, although none of these investigators have apparently seen my paper. I am quite sure that if they had they would have given it due recognition. A knowledge of its contents would have enabled them to interpret their results far more satisfactorily. Thus, Fantus<sup>2</sup> states that he is unable to explain why sodium bicarbonate alone has any antidotal value in cases of mercuric poisoning. The answer is that the metabolic changes taking place in the body are essentially oxidation-reduction processes. Hence, these numerous reducing substances react with the mercuric chloride at different but specific rates, which increase with decrease in concentration of acid; this decrease in the acid concentration is effected by the sodium bicarbonate. Another factor that has to be taken into account is the degree of complexity of the mercuric chloride, for the latter forms complex compounds<sup>3</sup> with other chlorides, and these complexes react exceedingly slowly<sup>4</sup> with reducing agents. Therefore, in order to obtain the best results, the condition of the system must be regulated accordingly. For example, every trace of acid must be previously neutralized if the reduction of the mercuric chloride to calomel by sodium phosphite is to be complete and instantaneous. To accomplish this without the introduction of an alkali, which is in itself poisonous, sodium salts of weak acids may be used. Sodium bicarbonate was found best for this purpose.

Theoretically a salt like sodium bicarbonate should be more effective in the neutralization of the acids in the system than sodium acetate because the reaction between sodium acetate and hydrochloric acid, formed in the reduction of mercuric chloride by reducing agents, is not complete, while the reaction between sodium bicarbonate and hydrochloric acid is instantaneous and quantitative, giving sodium chloride, carbon dioxide, and water. This is substantiated by experiment. The sodium chloride must, of course, be frequently removed together with the other products of the reaction by means of magnesium citrate or some similar substance, as the sodium chloride tends to retard the rate of the reduction of the mercuric chloride, owing to the formation of complex compounds previously mentioned.

Finally Fantus calls attention to the fact that a mixture of hydrogen peroxide and sodium hypophosphite is quite as efficient as sodium phosphite; but here again, he states that he is unable to explain "why hydrogen peroxide, an

\*Contribution from the Department of Chemistry of the University of California.

oxidizing agent, should increase the efficiency of a reducing agent like hypophosphite." A theoretical discussion of this belongs to the realm of theoretical chemistry. It may, however, be of interest to point out that stoichiometrically the reaction between sodium hypophosphite and mercuric chloride in the presence of hydrogen peroxide is identical with the reaction between sodium phosphite and mercuric chloride. This is shown by means of the following equations:



Moreover, hydrogen peroxide, as is well known, reacts with the blood, and is therefore very undesirable, especially for intravenous injections.

The publication of this paper at the present time is not merely to claim priority, for were it solely for this reason, it would not have been delayed until now. In fact, the two main reasons are, first, several physicians have requested me to publish the method of preparing the pure phosphorous acid as promised in my preliminary paper; and, second, I wish to suggest the possibility of administering rather large doses of mercuric compounds in infectious diseases, such as

syphilis, allowing the mercuric compound to remain in the system as long as possible and then removing the excess when necessary by means of sodium phosphite and sodium bicarbonate.

#### PREPARATION OF PURE PHOSPHOROUS ACID.

The method of preparing the acid is most conveniently described in connection with the figure.

The phosphorus trichloride is introduced into the separatory funnel, provided with a groove in the ground glass stopper for the admission of air, which permits the phosphorus trichloride to flow out into the flask via the stopcock. The phosphorus trichloride is allowed to flow into the water at such rate that there are never more than a few drops at the bottom of the flask. This is aided by passing a steady current of  $\text{CO}_2$  gas, the function of which is also to prevent oxidation of the phosphorous acid by excluding the air.

The  $\text{CO}_2$  entering the wash bottle on the right hand side is already purified, the function of this wash bottle being to supply moist  $\text{CO}_2$  for the subsequent removal of the last traces of  $\text{HCl}$ . The wash bottle on the left hand side serves merely as a gauge to regulate the suction at the exit of the gases, ( $\text{HCl}$ ,  $\text{CO}_2$ , and  $\text{H}_2\text{O}$ ). If the rate of drawing off of the gases is too slow, fumes of  $\text{HCl}$  gas will appear at the cork stopper of the flask. When this happens, the suction must be increased sufficiently to just prevent the fuming, otherwise, a small amount of air will be drawn into the flask. The cork, as well as the holes through which the separatory funnel and the two tubes pass, is lined with platinum foil to prevent the action of the moist  $\text{HCl}$  gas on the cork stopper and the subsequent contamination of the solution below. The cork thus protected is more satisfactory than all-glass connections, as the whole apparatus is not so rigid and therefore less fragile. Moreover, the flask is made of "Pyrex" glass which is best for the purpose, but which can not be safely sealed onto any other glass, and there are as yet no "Pyrex" separatory funnels on the market.

After the desired amount of phosphorus trichloride has been allowed to react with the water in the flask,\* (it is not necessary to cool the flask during the process) the wooden block upon which the flask rests (the flask is held in position by a clamp, not shown in the figure), is replaced by a water bath. The water is gradually warmed until its temperature is about the same as that of the melted paraffine bath by which the water bath is replaced. The paraffine bath is then gradually heated until its temperature is about  $180^\circ \text{C}$ . This temperature is maintained until the last traces of  $\text{HCl}$  are expelled. This is confirmed by replacing the wash bottle on the left hand side by another one containing a water solution of silver nitrate and nitric acid. The non-appearance of a white precipitate ( $\text{AgCl}$ ) shows that the  $\text{HCl}$  has been completely removed. The paraffine bath is then removed and the flask is allowed to cool down to about  $80^\circ \text{C}$ ., which is the melting point of phosphorous acid; during the cooling the current of  $\text{CO}_2$  is continued. The liquid acid is then poured into any

\*The amount of water to be used in the flask should be about 3 gram-molecular weight to one gram-molecular weight of phosphorus trichloride, or in the ratio of 54 grams of water to 137.5 grams of phosphorus trichloride, as is shown in the equation below. However, it is best to have the water in slight excess.





desired form and allowed to solidify, preferably in the form of sticks. The sticks so obtained are put into bottles provided with ground glass stoppers.

The acid obtained by this process is chiefly meta-phosphorous acid or dehydrated acid,  $\text{HPO}_2$ , the rest being orthophosphorous acid or normal phosphorous acid,  $\text{H}_3\text{PO}_3$ . Both of these acids dissolve readily in cold water.

#### PREPARATION OF SODIUM PHOSPHITE.

In my preliminary paper attention is called to the fact that the commercial sodium phosphite is a mixture of  $\text{NaH}_2\text{PO}_3$  and  $\text{Na}_2\text{HPO}_3$ , phosphate and other impurities,  $\text{NaH}_2\text{PO}_3$  being the chief constituent. This can be shown even in a qualitative way, by suspending the commercial sodium phosphite in warm water and adding a solution of  $\text{NaHCO}_3$ . A considerable effervescence of  $\text{CO}_2$  indicates the presence of  $\text{NaH}_2\text{PO}_3$ , while  $\text{Na}_2\text{HPO}_3$  barely reacts with sodium bicarbonate, as the third or last hydrogen atom in the  $\text{H}_3\text{PO}_3$  is not replaced to an appreciable extent by this method. It is, therefore, preferable to prepare the sodium phosphite solution from the pure solid stick-acid and pure  $\text{NaHCO}_3$  whenever needed. Pure  $\text{NaHCO}_3$  can be obtained from the Solvay Process Co., Syracuse, N. Y., through any reliable chemical concern. To test for the most common and undesirable impurity,  $\text{Na}_2\text{CO}_3$ , some of the sodium bicarbonate may be dissolved in cold water and a few drops of a solution of mercuric chloride added; a heavy reddish yellow precipitate indicates that considerable sodium carbonate is mixed with the sodium bicarbonate, while a mere opalescence or even a slight reddish brown precipitate shows that the sodium bicarbonate is fairly free from sodium carbonate.

Starting with the pure solid stick phosphorous acid and pure solid sodium bicarbonate, the following proportions by weight should be used for complete neutralization:  $\text{HPO}_2 + 3\text{NaHCO}_3 = \text{Na}_2\text{HPO}_3 + \text{NaHCO}_3 + 2\text{CO}_2 + \text{H}_2\text{O}$ , or in the proportion by weight of 1 to 4 of acid to bicarbonate, and the two dissolved in sufficient water to make about a 10 per cent solution.

It will be observed that this proportion of acid to sodium bicarbonate provides also for the subsequent neutralization of the last hydrogen atom after the  $\text{Na}_2\text{HPO}_3$  has been oxidized to  $\text{Na}_2\text{HPO}_4$  in the body, where the phosphite is administered as an antidote for mercuric chloride.

If the solution thus prepared is to be used for intravenous injections, it must, of course, be sterilized by filtration, and not by heating, as the sodium bicarbonate decomposes at about  $47^\circ \text{C}$ .

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<sup>4</sup>Linhart, G. A.: Ibid., 1915, xxxvii, 70.

References to the works of other investigators will be found in the article by Fantus, referred to above.

# TRAUMATIC RUPTURE OF THE HEART: REPORT OF A CASE WITH UNINJURED CHEST WALL

BY ELLIS KELLERT, M.D., ALBANY, N. Y.

**S**PONTANEOUS rupture of the heart is a not infrequent condition, and occurs more often than is commonly believed. Sudden death, in many instances ascribed to other causes, is found on postmortem examination to be due to coronary obstruction with subsequent infarction and rupture of a ventricle, or death may be caused by rupture of a fatty heart.

A few cases of cardiac rupture due to direct trauma applied to the chest have been described and in nearly all of these cases, fracture of the sternum or ribs with penetrating wounds of the heart were found. The case herein reported is of interest since it serves to illustrate that extensive trauma to the heart may occur with little or no injury to the thorax.

The accident took place near Ballston Spa. Mr. P. S., an Italian, aged 44 years, was engaged in digging in a sand bank. His fellow-workmen, a short distance removed, heard him cry out, and on turning about, found that the bank had caved in and the patient was surrounded up to the waist by a large quantity of sand. He was unconscious, but apparently alive. When dug out, he was dead. The above history is exceedingly meager, but no further information was obtainable.

At the autopsy, which was held eight days after death, the following conditions were found:

Bender Hygienic Laboratory No. A-15-26. The body, which has been embalmed, is that of a well built, muscular, white male adult. There is no evidence of injury to the skull. Over the upper anterior aspect of the chest, particularly on the left side, are five small abrasions measuring 0.5 to 2 cm. in diameter. Below the knees on both legs are several areas of abrasion, the largest of which, situated on the anterior surface of the right leg, measures 8x1 cm. There is no fracture of the bones of the extremities and no edema present.

**PERITONEUM.**—The peritoneal cavity contains approximately one liter of bloody formalized fluid. The viscera and their position appear normal.

**THORAX.**—Examination of sternum and ribs reveals no fractures. Diaphragm: right, 6th interspace; left, 7th rib.

**PLEURAL CAVITIES.**—The right pleural cavity is entirely obliterated by firm fibrous adhesions. The surfaces in the upper portion are hemorrhagic and blood stained. The lung is voluminous, but crepitant. Cut section is grayish in color and dry, but somewhat firm in the posterior portion of the lower lobe, which is reddish on cross section. There is a slight bloody suffusion in the apical region. The left pleural cavity contains a large amount of fluid blood and blood clot—approximately one liter of clot and 800 c.c. of bloody fluid. Portions of clotted blood are firmly adherent to the chest wall and dome of the diaphragm. The left lung is compressed into the upper portion of the chest and is negative on examination.

**PERICARDIAL CAVITY.**—On opening the pericardium there is found a large amount of firm blood clot, surrounding the heart and adherent to the pericardial surfaces. The lower left portion of the pericardial sac presents a ragged opening 4 cm. in diameter, which is filled with firm friable blood clot and which communicates with the left pleural cavity.

**HEART.**—It weighs 370 grams. It is found to the right of the midsternal line and is twisted slightly from left to right. There is a large amount of firm blood clot adherent to the anterior and right lateral surfaces. Near the apex, on the right posterior surface, is a ragged linear laceration of the heart wall measuring 5 cm. in length. The margins are deeply blood stained and there is free communication with both ventricles, the tear extending across the interventricular septum. Just beneath the right auricle are two minute openings in the fat and myocardium. In the left ventricular wall beneath the

auricular appendage is a transverse laceration in the heart muscle measuring 2.5 cm. Near the apex, situated mainly in the epicardial fat, are two circular communicating openings (embalmer's needle?). The myocardium is dull red in color. The right side of the heart appears contracted. The wall measures 7 mm. in thickness, but appears very thin near the laceration above described. Within the cavity there is found a small amount of friable blood clot. The papillary muscles are greatly lacerated, the surfaces rough and blood stained. The portion attached to the chordæ tendinæ is found free in the auricle. The tricuspid valve appears negative. The endocardium of the right auricle above the valve is markedly lacerated. The left ventricular wall measures 1.5 cm. to 2 cm. in thickness. In the left ventricle there is a very small amount of friable blood clot present. The papillary muscles have been torn from their attachments to the cardiac wall and their surfaces are very irregular, blood stained, and appear lacerated. The interventricular septum near the apex is greatly lacerated. The valve leaflets are intact. The aorta at its origin is separated through one-half the circumference, the margins being sharp, as though incised.

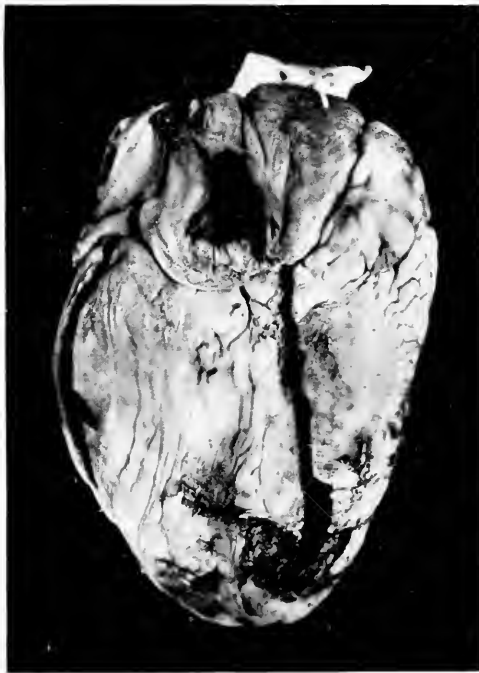


Fig. 1.—Heart, showing transverse rupture near apex.

GASTROINTESTINAL TRACT.—No injury or hemorrhage is found.

LIVER AND PANCREAS.—Appear normal.

SPLEEN.—It is enlarged and somewhat soft. The capsule is everywhere adherent to the peritoneal surfaces. Cut section is bright red in color. Surface scrapings are moderate. At the hilus is found a small spherical mass of splenic tissue.

KIDNEYS.—They are normal in size and weight, and are surrounded by a moderate amount of perinephritic fat. The capsule strips readily, exposing a markedly injected surface. Cut section is reddish-brown in color and the papillæ are congested. Bloody fluid escapes. In the outer portion of the cortex of the left kidney is a minute opening about which is fragmented kidney substance and from which blood escapes.

ADRENAL GLANDS.—They are normal.

BLADDER.—It is normal.

HISTOLOGY.—

*Heart.*—Slight amount of fatty infiltration of the right ventricular wall. Increased amount of pigment about the nuclei, which vary greatly in size and have blunt ends. An

occasional area of fibrosis is present and a rare focal accumulation of lymphocytes, endothelial cells and polynuclear leucocytes. Vacuolation is present in the muscle fibers.

*Lung.*—The vessels are distended and filled with blood. The capillaries in the alveolar walls are also dilated and filled with blood. Many red blood cells are found free in the alveoli.

*Spleen.*—The Malpighian corpuscles are small. There is a marked endothelial hyperplasia, and numerous eosinophiles are present.

*Kidney.*—Vessels prominent and filled with blood. Tubules negative. Glomerular tufts voluminous, and many are distended with blood. A small area in the capsule and adjacent cortex is suffused with blood.

*Aorta.*—Slight sclerosis and a few small areas of calcification in the media.

*Head.*—The brain was not examined.

**ANATOMICAL DIAGNOSIS.**—Multiple ruptures of the heart; hemopericardium; rupture of the pericardium; hemorrhagic effusion of the left pleural cavity; subpleural hemorrhage; chronic pleuritis; chronic interstitial myocarditis; acute congestion of lungs and kidneys; small rupture of the left kidney; chronic peritonitis; chronic splenitis and perisplenitis; accessory spleen; superficial abrasions.

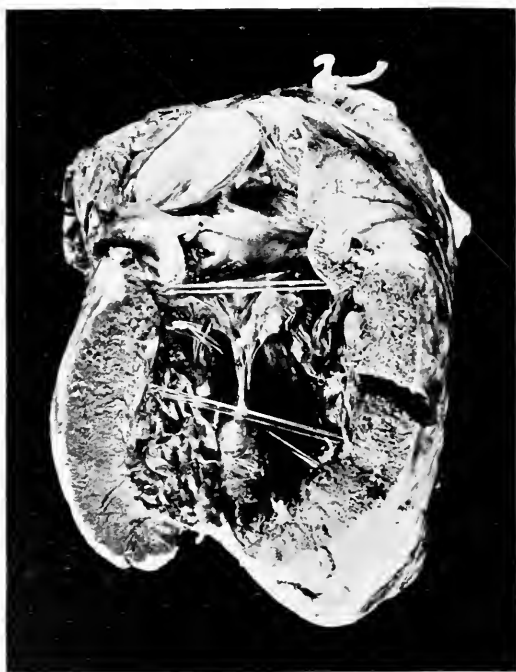


Fig. 2.—Heart, left ventricle. Note torn papillary muscle.

In the many cases of traumatic heart rupture already reported, severe injury to the chest wall has been the rule. A few exceptions are worthy of brief mention.

Dufour<sup>1</sup> reports the case of a girl thirteen years of age who underwent severe compression by a man and in addition presented stab wounds about the neck. A rupture of the right auricle was found, but no wound of the chest wall or pericardium.

McOscar and Voelcker<sup>2</sup> describe the case of a man who was run over by an empty wagon and died eight days later. At the necropsy there was found a transverse rupture of the ventricular septum one inch in length. No fractures of the ribs or sternum, and no lacerations of the abdominal viscera were present.

O'Neil<sup>3</sup> gives the autopsy findings in a boy aged nine years who was knocked down and jumped upon by other boys. He walked home, but died at the end of eight days. At the necropsy there was found a hemopericardium and a small rupture at the auriculoventricular junction of the left heart. All other organs were negative and since no mention is made of wounded chest or fractured ribs, it is assumed that none were present.

Kugel<sup>4</sup> described the case of a man forty-four years of age working in a cotton-spinning mill. He was killed by one of the heavy balls falling upon his chest and died forty-two hours later. At the autopsy there was found a hemopericardium and a sharp tear in the right ventricle. The heart was enlarged and fatty masses were present on the surface of both ventricles. There were no fractures or changes in the other organs.

Copeland<sup>5</sup> cites a case of ruptured aorta, occurring under circumstances simi-

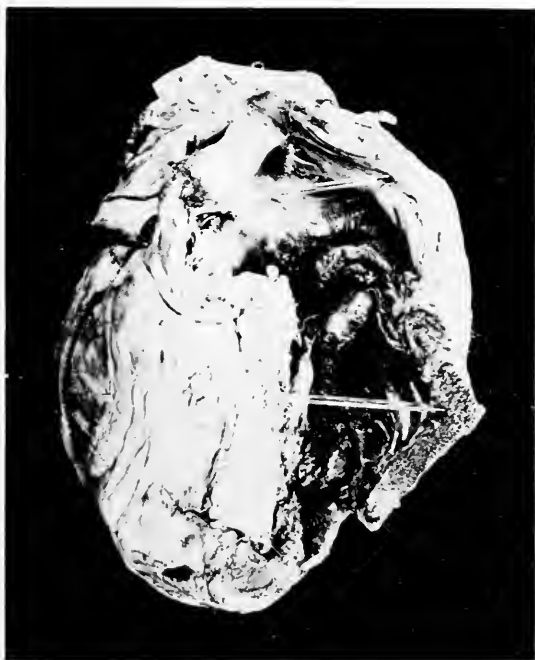


Fig. 3.—Heart, right ventricle, showing ruptured papillary muscle.

lar to the one here reported. His patient, a laborer aged forty years, was struck by falling earth but only the legs were covered. There was found a rupture of the descending aorta into the left pleural cavity which contained thirty-eight ounces of blood. He suggests a force acting in contrecoup.

Robertson<sup>6</sup> reports a man of forty-nine falling from a railway carriage and striking the ground with his left shoulder. He died in forty-five minutes. At the autopsy the pericardium was found intact, but a rent one and one-fourth inches long existed in front of the left auricle. There was no wound of the chest. Of course one must in this case consider the possibility of the rupture having occurred just prior to the fall although the situation in the auricle is very unusual for spontaneous rupture of the heart.

Gibbons<sup>7</sup> relates the following history. A coolie was struck over the chest as well as elsewhere with a bamboo stick. He fell to the ground and vomited, dying three hours later. A livid scratch over the right side of the chest was found but no penetrating wound. The heart showed a rupture at the apex, communicating with the right ventricle. The musculature at the point of rupture appeared very thin but no mention of aneurism is made.

The case of a man kicked on the chest by a horse is recorded by Hutchinson.<sup>8</sup> A hematoma precordia was produced, but the skin remained unbroken. The patient died fourteen hours later. There was a rupture at the apex of the right ventricle and the pericardium presented a tear anteriorly.

Groom<sup>9</sup> reports a lad sixteen years of age, was caught between the shaft of a pony cart and a wooden railing. Death occurred a month later and there was found a rupture in the posterior portion of the left ventricular wall one inch in length. A cardiac aneurism had developed at this point which later burst.

Peacock<sup>10</sup> relates the case of an athlete aged 25, who after a wrestling match became intoxicated and in a quarrel received a blow in the epigastrium from a clenched fist. He became unconscious and died in forty minutes. The autopsy disclosed a rupture of the right ventricle three and one-half inches in length, extending from the base to the apex. The valves were normal and no atheroma was present.

There are two possibilities in explanation of the lesion in the present instance. First, that the weight of the falling earth, which being soft and adaptable, caused equal pressure over the chest resulting in great compression and bursting of the heart, very much as though a rubber bag distended with fluid were compressed at its middle. To obtain such an effect without fracture of the sternum or ribs seems very unlikely. The second and more probable explanation is that of hydraulic pressure. The large quantity of sandy soil exerted such great pressure over the lower half of the body as to drive most of the blood out of the vessels. This produced sudden over-distention of the heart, which was probably dilated as a result of physical exertion, with consequent rupture at several points. That the force was a great one is indicated by the ruptured pericardium and hemorrhages in the lung. The pathologic changes in the heart, though not very marked, undoubtedly contributed to the severity of the injury.

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## ACUTE TUBERCULOUS PARIETAL ENDOCARDITIS\*

BY JOHN R. STARK, M.D., CINCINNATI, OHIO.

CARDIAC tuberculosis is not particularly uncommon. It appears in the form of myocardial, epicardial, valvular and mural infections. The myocardial disease may be of hematogenic origin when it is limited to that tissue or it may be the result of extension from epicardium or endocardium. Usually it is of epicardial origin. Epicardial tuberculosis is more frequent, so frequent, indeed, as to need no comment. Endocardial tuberculous lesions in the pure form are by far the most infrequent tuberculous cardiac lesion, and have been the subject of much study. In the less pure forms of endocardial involvement the evidence is that the bacilli reach the endocardium by way of the myocardial and subendocardial blood vessels, and therefore, as a rule, the myocardium is also involved. In the pure forms the infection must occur from bacilli present in the main blood stream which are able to become attached to the endothelial cells of the endocardium.

If so many tuberculous lesions are hematogenic in origin it must be true that tuberculous septicemia is not particularly uncommon; and if that be true, then the opportunities for endocardial infection must be numerous. It is perhaps the rapidity of the blood in the large open spaces of the heart that prevents lodgment of the bacteria, and it is the same factor, plus perhaps the presence of but small numbers of bacilli in the blood, that accounts for the rarity of pure parietal tuberculous endocarditis. In Marshall's<sup>1</sup> series of cases the tuberculous process seems to have appeared invariably beneath the endocardium, irrespective of the localization in the valves or in the mural endocardium.

Since Corvisart<sup>2</sup> reported his case of tuberculous pericarditis, and suggested the occurrence of tuberculous endocarditis, considerable study has attached to these lesions. The major amount of these various contributions has been reviewed by Norris<sup>3</sup> who credits Corvisart with an account of mitral valvular tuberculosis. But so far as I can discover, Corvisart's case was one of pulmonary tuberculosis complicated with vegetative mitral endocarditis, for, to use his own words (in translation), "the mitral valves and semilunar of the aorta were covered with vegetations exactly like venereal warts observed upon the glans and prepuce of persons affected with syphilis" (pages 180-181). But Corvisart does describe a case of tuberculous pericarditis, and he does it very definitely.

In the entire published series of cases of cardiac tuberculosis there appear, so far as I can discover, but two in which the lesion was definitely in the endocardium,—those of Schultze<sup>4</sup> and Rheinhard.<sup>5</sup> Only in the report of Rheinhard, however, are there definite pictorial descriptions of the lesions. Benda,<sup>6</sup> in discussing subendocardial or myocardial tubercles and in differentiating between true endocardial lesions, laid down the rule that the structure of the internal elastic lamella, would serve as the best criterion. If this were damaged, the evidence led in the direction of a primary subendocardial lesion.

\*From the Mary M. Emery Department of Pathology of the University of Cincinnati, and the Pathologic Institute of the Cincinnati General Hospital.

While I do not agree completely with the rule of Benda, the case which is to be reported conforms to it and, therefore, appears to be the third authentic case of primary acute parietal or mural tuberculous endocarditis to be recorded.

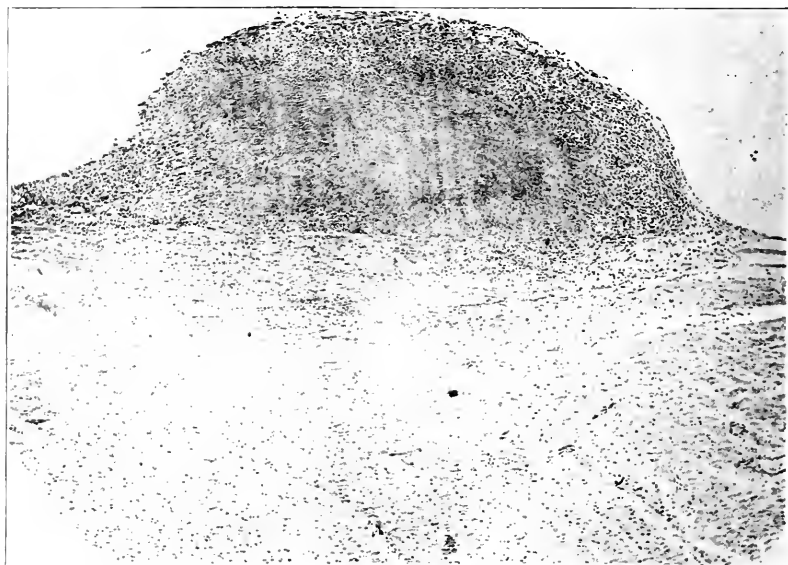


Fig. 1.—The entire tuberculous nodule with caseating center and intact endothelium, except at one point, situated within the endocardium. It shows the typical base consisting of epithelioid and round cells with fairly numerous typical giant cells. The endothelial lesion is probably an artifact.



Fig. 2.—A resorcin-fuchsin specimen to show that the caseating mass is entirely above the elastica interna. There is very slight evidence of subendocardial reaction visible at only one point in the myocardium.



## CLINICAL NOTES.

The patient, J. M., Hospital No. A-7524, was admitted into the Cincinnati General Hospital on the Orthopedic Service, on November 11, 1916, and died March 23, 1917.

Unfortunately in the hospital records there was no history of the past illnesses or present condition of the patient who was a negro porter aged 32 years. The record consisted of daily notes as to the clinical condition of the patient, operative procedures, x-ray reports, and urinalyses. The important features gleaned from these meager notes are that an x-ray examination taken a few days after admission on November 14, "showed gross destruction of the body of the first lumbar vertebra and also some changes in the body of the twelfth dorsal." On this same day the patient was operated and two large abscess cavities located somewhere along the spine were opened and drained. He gradually improved from this time on and January 11, 1917, a cast was applied and he was allowed up in a chair for a short time. A urinalysis on January 31 showed a cloudy, amber, alkaline urine with a specific gravity of 1012, albumen positive, and microscopically many pus cells, hyaline and granular casts but no blood. The next clinical note recorded about two months later, the day of death, states: "The patient has been gradually growing weaker. Tuberculous meningitis set in 3/18/17. The patient became violent, then went into coma 3/22/17, and died 3/23/17 at 9:10 A.M."

*Clinical Diagnosis.*—Dorsal and lumbar Potts; tuberculous meningitis.

With this we must be content.

## AUTOPSY PROTOCOL.

The body was that of a greatly emaciated colored man of about 35 years of age. The body was still warm and postmortem rigidity had not yet set in. The muscles of the right arm were more atrophic than those of the left. There was no noticeable difference in the muscles of the legs. On the back, over the 11th and 12th thoracic and first two lumbar vertebrae, there was a superficial abrasion, and in the region of the 12th vertebra there was a gibbus. The gibbus, however, was also made up of the 11th and 12th and the 1st and 2nd vertebrae. Below over the sacrum, there was a strip of adhesive tape covering a small sinus from which there oozed a quantity of thick yellowish pus. To the left of the spine, beneath the 12th rib there was a larger sinus from which a similar purulent material poured out. The pupils were equal and slightly dilated. The teeth were in fair condition and there was only a moderate pyorrhea. There was marked anemia of all the mucous membranes. At the base of the neck there was a symmetrical swelling as from a goitrous enlargement. There was no peripheral glandular enlargement except for a few small pea-sized inguinal glands. The musculature over the chest and abdomen was greatly diminished; there was no subcutaneous fat.

When the sternum was removed, the lungs partially collapsed. There were no pleural adhesions nor any fluid in the pleural cavities. There was about 25 c.c. of clear straw-colored fluid in the pericardial cavity. The lungs upon removal, crepitated throughout. The apices were not scarred and there were not even interlobar adhesions. On section, the lungs were perfectly healthy throughout.

The heart was slightly enlarged and extremely pale. The left side was rather firmly contracted while the entire right side was very flabby and dilated. There was no valvular abnormality throughout. The aorta was perfectly clean. Just beneath the endocardium of the left ventricle about 3 cm. from the apex on top of a columnus carnea there was a somewhat whitish, slightly firm, raised nodule about 2 mm. in diameter which on section appeared homogeneous and was perhaps a tubercle. The myocardium itself was pale and presented a few areas of fibrous bands. These were particularly marked in the tips of the papillary muscles.

Upon opening the abdomen there was no excess of fluid. The peritoneum was shiny throughout and the omentum lay curled up over the transverse colon. The intestines were thin and shrunken, the appendix was *in situ* and free from adhesions. Upon removal, the entire gastrointestinal tract was healthy except for one small ulcerating tuberculous mass about 1 cm. in size, in the ascending colon just above the cecum.

The spleen was enlarged. The capsule was smooth and shiny, but beneath it there could be seen numerous large and small whitish areas. On section, the spleen showed numerous milary and conglomerate tubercles, the largest not over 3 mm. in size. The parenchyma of this organ, except for this, showed no change. The liver was pale and beneath its capsule could also be seen a few milary tubercles. The organ cut with increased difficulty and throughout it were seen occasional milary tubercles. The organ

itself was extremely pale and cloudy, but its consistency was generally increased, yet showed no gross evidence of fibrous tissue. The gall bladder was free from adhesions and contained a dark clear bile. The right kidney was enlarged, and of a pale red color. It cut with increased resistance and showed numerous conglomerate tubercles, none measur-

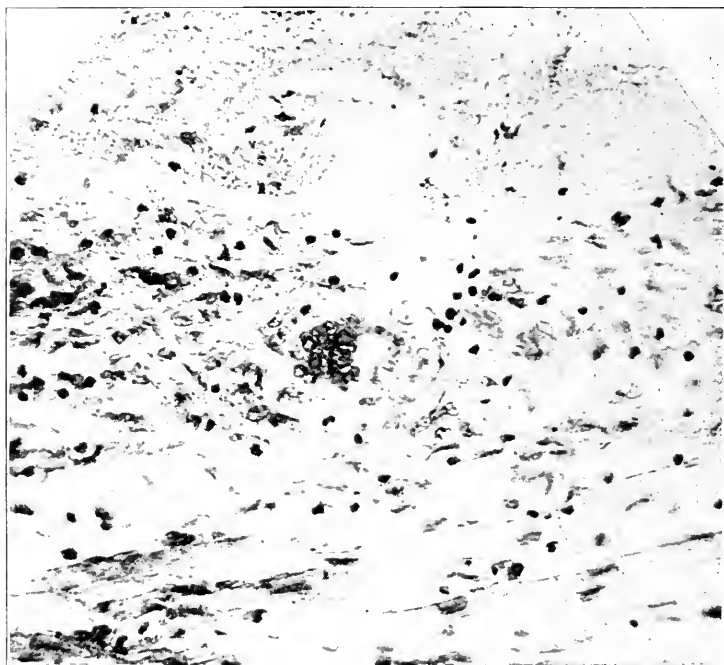


Fig. 3.—A larger magnification of the base showing two typical giant cells with numerous round and epithelioid cells.

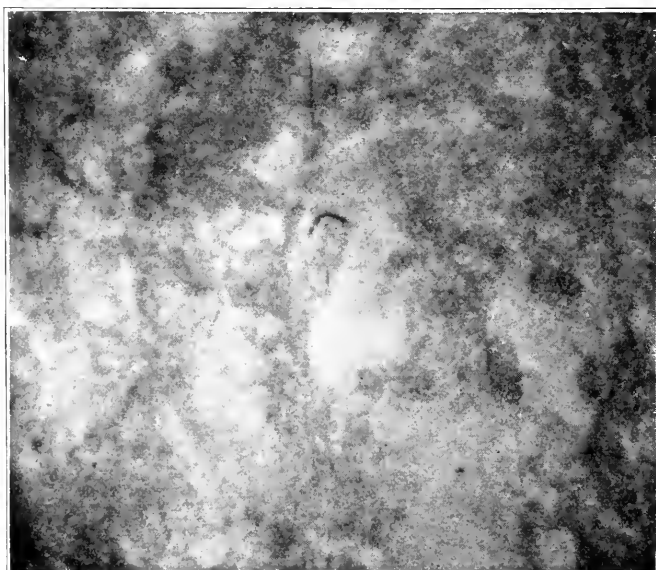


Fig. 4.—A typical acid-fast bacillus of the slightly curved type lying in the richly cellular area. These bacilli were quite numerous but were seldom found lying parallel with the plane of section, hence the hazy, indistinct outline in the photomicrograph. They were usually curved and irregular and possibly were of the branching type, as described by Woolley<sup>7</sup> in "acute tuberculous endoarteritis."

ing more than 3 mm. in size. The cortex was thickened, not well marked off from the medulla, and had a pale pinkish color. There was no evidence of Malpighian corpuscles. The capsule stripped easily, leaving a smooth surface. The stellate veins were not injected. The right ureter was distended to about the size of a pencil to its entrance in the bladder. The pelvis was apparently healthy. The left kidney was tremendously enlarged. It was adherent to the psoas muscle behind and was removed with great difficulty. In removing it the kidney substance was torn and a considerable amount of watery yellowish pus was expressed from an opening on the posterior surface of the organ which extended into the psoas muscle. The kidney itself showed a very thin cortex in which were numerous miliary and conglomerate tubercles. The entire medulla and pelvis were replaced by a large tuberculous abscess cavity which was somewhat cauliflower in shape and extended throughout the kidney. The left ureter was obliterated by a firm white glistening columnar mass of tuberculous tissue about  $1\frac{1}{2}$  cm. in diameter, which extended from the pelvis to the bladder. No lumen was discernible. The bladder was distended to about two inches above the pubis, and was filled with a very cloudy turbid amber-



Fig. 5.—A straight beaded acid-fast bacillus in the endocardial tubercle.

colored urine. Many flocculent white masses floating in the urine contained calcium salts. The mucosa was of a pale white color and was covered by these flocculent masses. The bladder wall showed numerous miliary tubercles, but no ulceration. At the opening of the prostatic urethra, the entire mucosa was ulcerated and covered with tuberculous granulations. The prostate was small and apparently healthy. After removal of all the abdominal viscera, it was seen that the body of the 12th dorsal vertebra was eroded and softened on the left side and in the region of the pedicle the bone was completely eroded and destroyed so that the finger could be introduced into the spinal canal.

Permission was not obtained to open the calvarium or explore the spinal canal further. The lymph nodes along the course of the abdominal aorta, more particularly upon the left side, and extending down into the pelvis along the iliac vessels, were greatly enlarged, firm, and, upon section, appeared to be completely replaced by tuberculous material. The thyroid was enlarged, the right lobe slightly more so than the left. It cut with rather increased resistance and was extremely pale. In the upper pole of the right lobe there was a small, white, fine stellate area possibly tuberculous, but appearing more like a mass of fibrous tissue. There were no cysts.

*Anatomic Diagnosis.*—Chronic tuberculous spondylitis; psoas abscess; chronic tuberculous nephritis; chronic tuberculosis of the retroperitoneal glands; metastatic tubercles of the liver, spleen, right kidney, adrenals and possibly the myocardium; tuberculous colitis; chronic tuberculous cystitis and ureteritis; amyloidosis of the liver and kidney; cardiac dilatation; myocardial fibrosis; hypertrophied thyroid.

Realizing at the time of autopsy the great infrequency of endocardial tuberculosis, and not appreciating in the gross specimen that the tubercle was entirely in the endocardium, the anatomical diagnosis and necropsy report were first conjectured, but later proved by microscopical sections.

Although this case, at first glance, might be considered one of generalized miliary tuberculosis, yet it presents many interesting features. The entire absence of any pulmonary or peritoneal involvement is truly unusual. The kidney lesion was evidently one of extension from the tuberculous spondylitis. Involvement of spleen and liver were minimal.

The accepted theory of acute generalized miliary tuberculosis is that a tuberculous focus adjoining some blood vessel erodes the vessel wall and the bacteria are disseminated throughout the body. In this case we have another source for the scattering of the bacilli. Had the patient lived longer an ulcerated area might have been met with together with a greater involvement of the abdominal organs. However, since tubercle bacilli are accredited with the power of traversing intact intestinal mucous membrane, it seems entirely possible that the existing foci might have been due, in part at least, to the passage of the bacteria from the caseating mass through the intact endothelium.

There are perhaps forty cases of tuberculous endocarditis described, and only three of true tuberculous parietal endocarditis. This same relation between vegetative and mural endocarditis of other bacterial origin is met with. This fact is very suggestive of the infrequency of implantation endocarditis as compared with the more accepted embolic theory.

#### CONCLUSIONS.

1. This is the third recorded case of tuberculosis of the parietal endocardium.
2. Here is definite evidence of the implantation theory of endocarditis, however infrequent.

In conclusion, I wish to thank most sincerely Dr. P. G. Woolley, Director of the Pathologic Institute of the Cincinnati General Hospital, for his kindness and encouragement in the above, and Mr. O. Haude, for his energy and skill in procuring these most excellent sections and photomicrographs.

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# LABORATORY METHODS

## DESCRIPTION OF AND PLANS FOR THE INSTALLATION OF A BLAST AND VACUUM PUMP\*

BY W. M. H. WELKER, CHICAGO, ILL.

### INTRODUCTION.

THE form of installation applied to the blast and vacuum apparatus in this laboratory has frequently been the subject of favorable comment on the part of visitors. A number of requests have been received for scale drawings and a general description of the installation. It seemed probable, therefore, that the publication of a description accompanied by working drawings, might be of benefit to the workers in laboratories not supplied with blast and suction from a central plant.

The machine used is the Crowell Rotary Blast and Vacuum Pump, catalogued by most of the apparatus supply houses. The installation described, is the result of six years of experimentation with this form of machine. The advantages of this type are as follows: Its original cost is comparatively low; the operating and maintenance costs are low; it is noiseless in operation; it is possible to secure pressure up to 40 pounds per square inch and vacuum down to 29 inches of mercury; and blast and suction within certain limits are obtainable simultaneously.

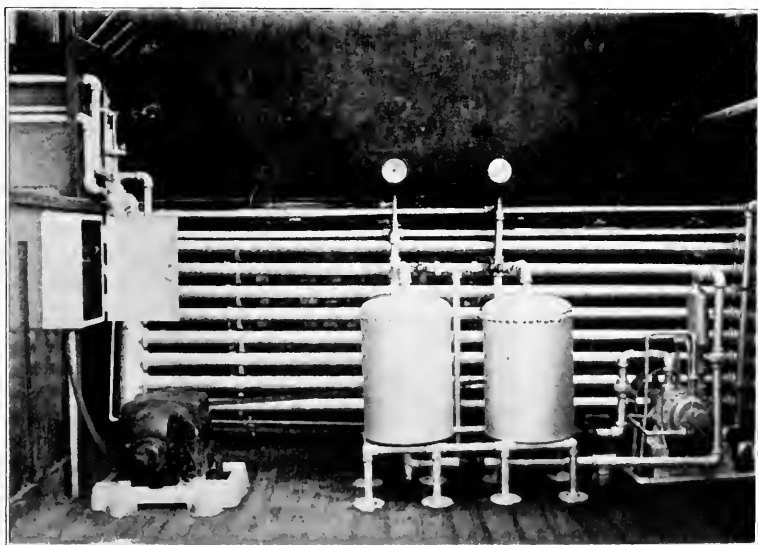


Fig. 1.—Photograph of the installation in the Laboratory of Physiological Chemistry, College of Medicine, University of Illinois.

\*From the Laboratory of Physiological Chemistry, College of Medicine, University of Illinois.

## USES.

In this laboratory, the apparatus is used for running blast lamps, stirring solutions, handling liquids, drying by air current, artificial respiration (with interruptor interposed,) vacuum distillation, aeration, and suction filtration. The liquids that are handled by compressed air include a strong solution of alkali for the Kjeldahl distillation work. The arrangement consists of a delivery tube which runs to the bottom of the bottle serving as container for the solution. A "T" tube serves to connect the pressure line with the container, the third opening being closed with the finger when it is desired to transfer liquid from the container to another vessel. Special mention is made of this point because con-

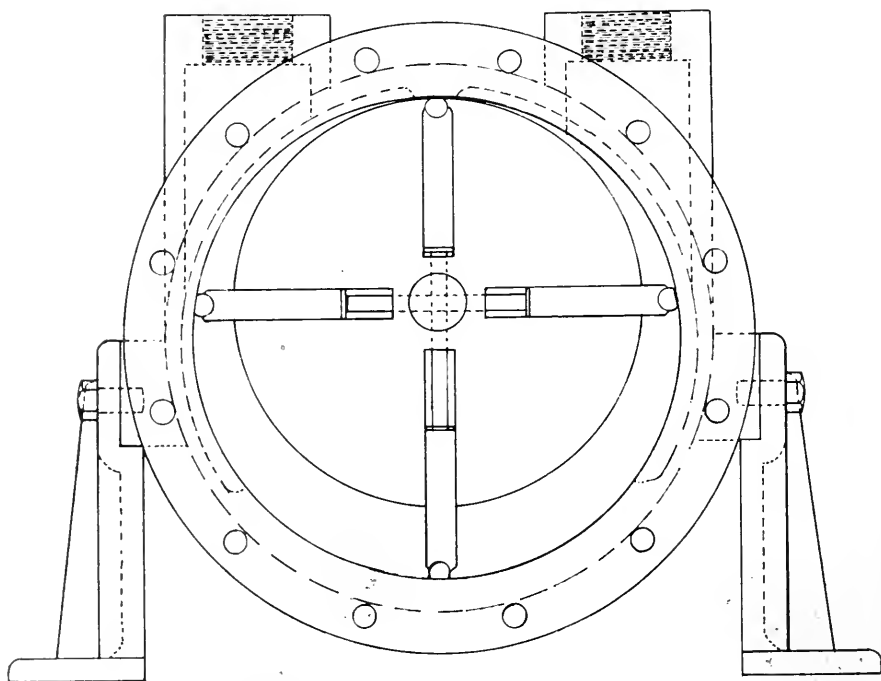


Fig. 2.—The plan of interior construction of the Crowell Rotary Blast and Vacuum Pump.  
(Courtesy of The Crowell Mfg. Co.)

siderable effort has been given by laboratory workers, to the construction of special apparatus for the handling of this solution.

## INSTALLATION.

One of the first difficulties encountered in the installation of this machine originated in the fact that the smaller alternating current motors obtainable on the market are built to give a speed of 1800 R.P.M. The smallest pulley that manufacturers recommend for this motor is  $2\frac{1}{2}$  inches in diameter. This necessitates a 9 inch pulley on the machine to give it the operating speed suggested by its manufacturer. The 9 inch pulley dips below the level of the frame of the machine and an idler had to be placed under the belt to prevent it from scraping on the latter. This idler caused considerable trouble and was noisy. The diffi-

culty was finally overcome by placing hardwood blocks between the machine and its frame, thus raising the bottom of the pulley sufficiently for the belt to clear the frame.

Brass lever stopcocks are used for regulating the pressure and vacuum in the tanks, instead of the adjustable spring valves furnished by the manufacturers.

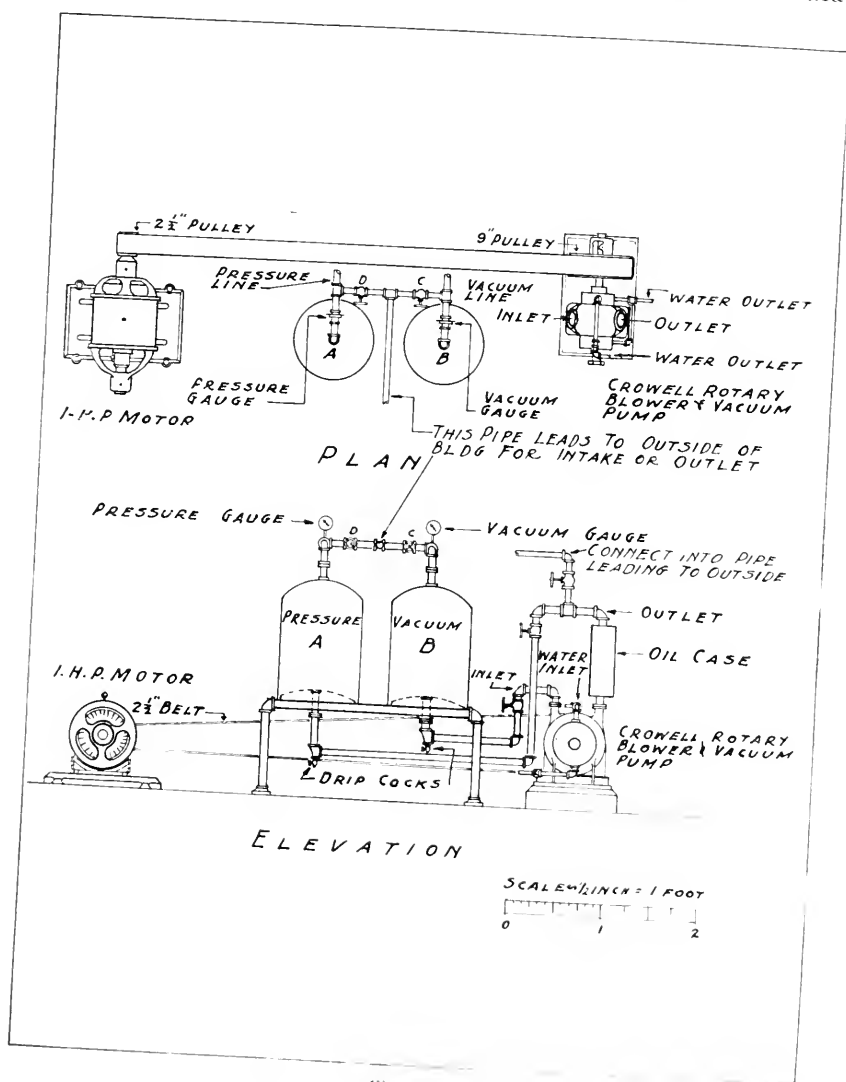


Fig. 3.

Adjustments can be more easily and accurately made with the lever stopcock than with the spring valve. Wherever gate valves appear in the plan, brass lever stopcocks are actually used in the installation. The escape of excess compressed air through either spring valve or stopcock is a noisy operation. The same is true of the operation of either of these valves on the vacuum tank. The noise was completely eliminated by connecting with the blast outlet "D" and vacuum intake "C," a common pipe leading to the outside of the building.

By closing the respective stopcock on the line from the machine to the tank, compressed air or vacuum may be stored so that it can be used after the pump has been stopped. This method is especially well adapted for the vacuum filtration of difficultly filterable solutions.

In the vacuum distillation of ether, alcohol, and liquids of similar character, explosive mixtures will be carried into the pressure tank. To prevent this, a crossover with stopcock has been placed between the line from the machine to the pressure tank and the line to the outside of the building. By opening this stop-

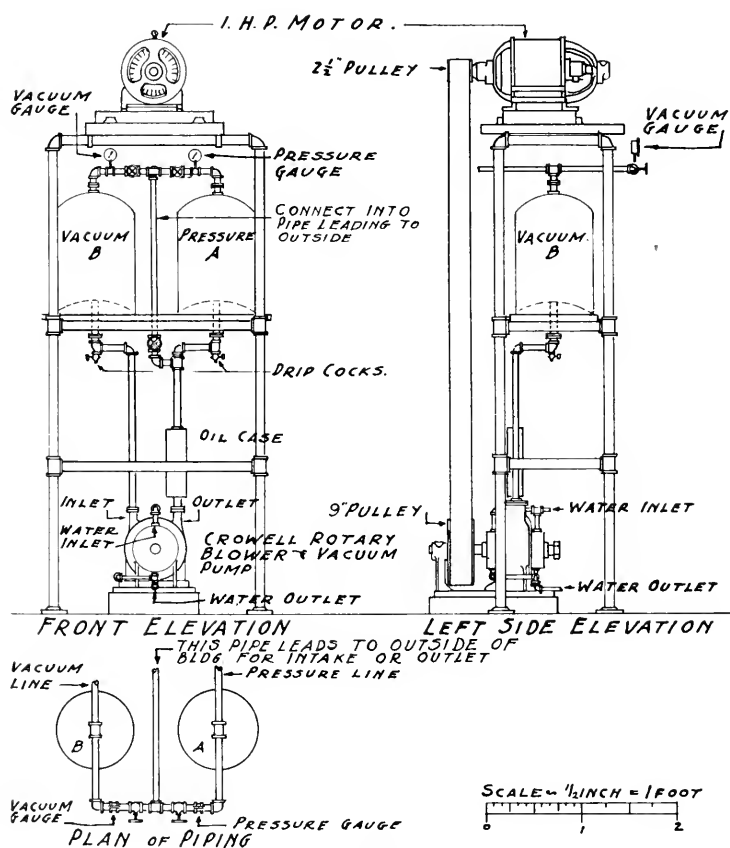


Fig. 4.

cock and closing the stopcock in the line leading to the tank and also the stopcock "D," the mixture of explosive gases is conducted directly to the outside of the building.

I am indebted to Mr. Tracy, formerly of this staff, for assistance in some of the experimental work on the installation. I am indebted also to Professor White, supervising architect of the University, for the preparation of the scale drawings, and for the suggestion of the vertical form of installation. Where floor space is limited, this plan of installation would be preferable to the horizontal one.



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## EDITORIALS

### *Dried Tetanus Antitoxin*

SOME months ago the Committee on Medicine and Hygiene of the National Research Council took up this subject, having learned that such a preparation was being used in the German army. It turns out that Robertson, of the University of Minnesota, was at work with Aschoff in Freiberg, Germany, on the preparation of dried tetanus antitoxin during the first year of the war. Robertson writes as follows:

"I may state that we felt that the results of our experiments would justify us in trying to produce a first-aid method of administering tetanus antitoxin by drying it on pieces of gauze or cotton and carrying it in this dried condition in the first-aid package. It was found that if these dried pads were placed on wounds they gave protection to animals which had received from two to five times the minimum lethal dose of tetanus toxin. During the past two years I have been working in my spare time on the dose of antitoxin necessary for prophylactic purposes. While my experimentations are not finished, they point to the possibility of considerable reduction in dosage with full and adequate amount of defense provided. The short time that the antitoxin immunization

persists renders, in a great many cases, a second dose necessary in any event, and the smaller doses seem to be fully as sufficient as larger ones."

It is not supposed that the dried antitoxin will do away with the necessity of antitoxin injections. A bit of the cotton containing the dried antitoxin is moistened with a sterile water or salt solution and applied to the wound in the first-aid dressing. Then, as soon as possible, the antitoxin injection is given.

—I. C. I.

### *Blood Alkalinity and Cancer*

ANY procedure which will make possible the more certain diagnosis of cancer, is valuable. There are times when a cancer is suspected in a patient at a period during which operative procedures might be carried out with success, and yet in such patients there is a reasonable doubt which leads to a diagnosis of some less serious condition which is not to be treated radically.

Menten<sup>1</sup> in a study of blood alkalinity (i.e., the hydrogen-ion concentration) has reached the general conclusion that while the alkalinity of the blood serum may be increased or diminished in various diseases, yet almost without exception the cases of malignant disease, that is, cases of cancer of the skin and internal organs, and of sarcoma, show a characteristic change in the reaction of the blood serum. This change consists in a marked increase in the alkalinity of the serum. In contrast to these findings are the results obtained in cases of tuberculosis, rheumatism, pemphigus, nephritis and heart lesions accompanied by dyspnea in all of which the acidity of the serum is increased. The increase in alkalinity of the serum in malignancy is so constant and of so marked a character when the growth occurs in the internal organs that it is believed that it may furnish an additional diagnostic aid of value, particularly, in early stages.

Menten used the gas chain method in determining the hydrogen-ion concentration of the blood, a method which is not usable by the ordinary laboratory man or by the clinician who makes his own determinations, but it is entirely possible that one of the simpler methods in vogue may be of rapid clinical value.

It is interesting to speculate upon the relation of the alkalinity to malignant growth.<sup>1</sup> Is it cause or effect? Menten mentions a case in which, after the administration of sodium bicarbonate, the rate of growth of a cancer was hastened.

High alkalinity is not a certain indication of cancer, but its presence in a suspected case is an added reason for a positive diagnosis. The method therefore is like practically all other laboratory tests, merely an aid, perhaps a very valuable one, to the careful clinician. To the careless one it is apt to be a hindrance, or to be misleading. Albumin and casts do not necessarily mean nephritis. These appear in other conditions,—but their presence, under certain conditions, is highly suggestive.

—P. G. W.

<sup>1</sup>Menten: Jour. Cancer Research, 1917, ii, 179.

# *The Journal of Laboratory and Clinical Medicine*

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NO. 11.

## ORIGINAL ARTICLES

### THE PRINCIPLES INVOLVED IN THE ECONOMIC READJUSTMENT OF DIETARIES\*

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THE basic standard of diet is its energy value. The energy value of the foodstuffs is the currency of dietetics. Just as in the barter and trade of commerce some common basis of exchange is necessary, something, that is to say, having a standard value with which the value of all other things can be compared, and by the use of which they can be bought and sold; so with food, in order to exchange one foodstuff for another in the dietary, some standard of relative values—some food currency—must be used. The basis of monetary currency is the dollar; that of food is the calorie.

This does not necessarily mean that the calorie, although itself a fixed value, is always of exactly the same dietetic importance, for just as in the monetary system the purchasing power of the gold dollar may vary in different countries, so in dietetics may the relative importance of the calorie vary with the foodstuff which supplies it. Thus, the ration of one individual may be quite inadequate or may be harmful for another. "Ae man's meat's anither man's poison." Quite apart from gratification of appetite, which, however, is a most important factor in food assimilation, the food consumed by each individual must be properly adjusted to meet his peculiar requirements. Besides its caloric value, therefore, certain other values for foods must be taken into consideration, but at the outset attention is called to the calorie.

A calorie is the unit of energy. It can be used to measure the expenditure of energy, whether this occurs as heat, or as mechanical work, or as electrical discharge, or as chemical reaction. To make this plain, consider the relationship between the expenditure of energy as motion and as heat in the case of a

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steam engine. When the engine is at rest, all the energy is dissipated as heat, but when it works, some of this heat takes the form of movement, the sum total of energy expenditure being the same whether the engine is standing still or is moving. The source of the energy given out by the engine is obviously the fuel which is burned in the furnace. Turning now to the animal body, the fuel which it burns is the food. Everything capable of burning outside the body is not suitable for the animal fuel, but only those substances which can be acted on by special types of reagents present in the animal, called enzymes or ferments, which have the function of loosening up and breaking apart into smaller molecules the large complex molecules of which food is composed. The smaller molecules then unite with the oxygen of the blood and become burned or oxidized and liberate their energy. The foods belong to three classes of organic substances: fats, carbohydrates, and proteins.

In order to find out how much energy is available in a fuel, it is necessary to burn it and measure the amount of heat which it gives out in the process. The measurement of the amount of heat is not, however, so very easy a matter to comprehend. It is not a question merely of increase in temperature, for it is obvious that there must be very different *amounts* of heat in an ounce and in a pint of boiling water: placed side by side in similar vessels the ounce of water will cool off much more quickly than the pint. This simple example indicates a basis upon which we may measure heat, namely, as the temperature multiplied by the volume or mass of water. The unit of such measurement is called a calorie, which is defined as the amount of heat required to raise the temperature of 1 kg. of water ( $2\frac{1}{4}$  lb.) through  $1^{\circ}$  C., or, roughly, 1 lb.  $\text{H}_2\text{O}$ ,  $4^{\circ}\text{F}$ . In order to measure the caloric value of a fuel or foodstuff, we must, therefore, ascertain the degrees of temperature through which a known volume of water is raised by burning a weighed amount of substance in such a way that all the heat which it gives out is taken up by the water.

The apparatus for doing this is called a calorimeter, which consists, in principle, of a stout iron chamber, or bomb, into which a weighed amount of the fuel, or food, is placed and then filled with compressed oxygen. The bomb is tightly closed, placed in an outer vessel containing a known volume of water at a known temperature, and the fuel or food set on fire by an electric current. When 1 gm. (15 gr.) quantities of each of the three foodstuffs are burned in the calorimeter, the values are: protein, about 5.5; fat, 9.3; carbohydrate, 4.1. That means, in the case of fat, for example, that if 1 gm. be burned in the chamber of a calorimeter, which, it will be assumed, contains 1000 gr. water, an increase of temperature amounting to  $9.3^{\circ}$  C. will be observed. No matter how slowly or quickly the given material burns, the caloric value is always the same.

To compute the total energy taken into the body, all we have to do is to weigh each article of food, ascertain from the numerous dietary tables readily available in textbooks, encyclopedias, government bulletins, etc., how much fat, protein and carbohydrate it contains, and then multiply each of these by its proper caloric value and add the results together. This gives us the caloric value available when the foodstuffs are completely burned. But some of the food is not entirely oxidized in the animal body, so that to determine the physiologic caloric value, we must subtract from the crude value the caloric

value of the unused portion given out with the excreta. In the case of fats and most carbohydrates, the subtraction is very small if digestion and assimilation be in good order. It is considerable, however, in the case of protein, bringing the actual caloric value of this foodstuff down to 4.1, the same as for carbohydrates.

So far we have gone on the assumption that the caloric value of the foods will be the same whether they are quickly burned or only slowly used up in the life processes of the animal body. Obviously, however, before dietetics can be considered to rest on any scientific basis, indisputable evidence that such is really the case, must be furnished. The energy output of animals must be measured to show that it balances up exactly with the energy value of the food which has meanwhile been consumed. One of the greatest accomplishments of modern physiologic science is the fact that it should have been possible to do this very thing. For this purpose a calorimeter is again used, but modified so that it may be constantly ventilated to prevent suffocation of the animal. This is called a respiration calorimeter. It consists of an air-tight chamber with double walls, in the space between which is an ingenious device by which the temperature of the air of the space is regulated so as to be exactly the same as that of the chamber itself, thus preventing entirely any loss of heat. The heat given off by an animal placed in the calorimeter is measured by observing the change in temperature produced in a known volume of water passed through radiators in the chamber, that which is meanwhile employed to evaporate the water given out in the expired air and sweat being readily computed by collecting this vapor in suitable absorption bottles (see p. 746) placed in the course of the ventilating tubes. Calorimeters have been constructed by Atwater, Benedict and Graham Lusk, in which a man can live comfortably for long periods of time.

The above comparison can not be made by merely measuring the food the animal eats, because the food is not necessarily consumed immediately after it is taken; it must first of all become assimilated in the body, and this takes some time. Months indeed may elapse between the time that a food is eaten and that when it is ultimately oxidized to yield energy. It is on this account that a starving animal may go on yielding energy; he lives on the foodstuffs which have been incorporated with his body; he feeds on his own tissues. The problem is not so simple as in the case of a steam engine, where we may compare the energy output with the fuel consumption.

How, then, is it known that each of the foodstuffs gives out as heat and other forms of energy the same amount of heat which it produces when burned in a calorimeter? The rate at which combustion is proceeding in the body must be measured by measuring the products of the combustion.

To understand properly the principle upon which such a measurement depends, turn for a moment to the conditions obtaining when a piece of sugar or fat is burned outside the body. As the combustion proceeds, carbonic acid and water are given off because of the union of the oxygen of the air with the carbon and hydrogen, of which elements, along with some intramolecular oxygen, either of these foodstuffs is composed. By the oxidation, energy is liberated as heat, so that the number of calories given out by the burning process

is directly proportional to the amount of water and carbon dioxide meanwhile produced and the amount of oxygen used up. It is, chemically, a comparatively easy matter to measure the amounts of these combustion products. The carbonic acid is measured by finding how much alkali it can change into carbonate and by leading the air which contains it as vapor through substances, such as concentrated  $\text{H}_2\text{SO}_4$ , which absorb it. The analysis of proteins is more complicated on account of the fact that, besides carbon, hydrogen, and oxygen, they contain nitrogen. By suitable chemical processes this nitrogen has either to be dislodged from them, or converted into its hydrogen compound, ammonia, which is then very easily measured.

Let us see now how these methods of the chemist may be employed to measure the products of combustion in the case of an animal. For this purpose the chamber of the calorimeter is connected with an air-tight system of wide-bore tubes, along which an air current is made to circulate by means of a rotary blower or fan. The water and carbon dioxide given off by the animal are caught in so-called absorption bottles, inserted in the system and containing suitable reagents to combine with the gases. The oxygen consumed by the process of combustion causes the volume of air in the system to shrink, but just as quickly as it does so, as indicated by a gauge, fresh oxygen is discharged into the system from a cylinder of the gas. In a chemical analysis, as we stated above, the water value, as well as that of carbon dioxide, is used in calculating the composition of the substance burned. In a metabolism experiment, however, as the above physiologic method is called, the water excretion is not of much value as a means of determining the amount of combustion, because of the fact that relatively large and inconstant amounts of it are taken with the food, and the body at different times contains varying amounts of it. The carbon dioxide excretion, taken along with the oxygen intake, is the important criterion. In short, then, by measuring the carbon dioxide absorbed by the absorption bottles and the fresh oxygen that has to be delivered into the system to keep the volume constant, one can tell exactly how much material containing carbon and hydrogen is being oxidized in the body.

When we know how much carbon has been oxidized, we can not tell how much of it came from protein, fat, or carbohydrate, because all three contain it; and the energy value of each being different, we can not compute how many calories have *in toto* been liberated by the combustion process. To find out what foodstuff was actually consumed, some other excretory product that is peculiar to one or the other of the foodstuffs must be observed. In the case of protein, this is made possible by the fact that the amount of nitrogen which the animal excretes during his stay in the chamber can readily be measured. Having found from the nitrogen excretion how much protein must have become used up (by multiplying grams of nitrogen by 6.25), we may then calculate the amount of carbon contained in this amount of protein and subtract it from the total carbon which must have been burnt to produce the carbon dioxide. The remainder is the carbon of the fat and carbohydrate that have been burnt. The relative amounts of these two can then be computed from a knowledge of the relationship of the oxygen absorption to the carbon dioxide excretion—the so-called respiratory quotient.

Having thus become acquainted with the general principles of which the necessary measurements are made, the results are next to be considered. These are among the greatest achievements of modern medical science, for when the measurements are properly made, the caloric output as directly measured corresponds exactly with that calculated by multiplying the amount of each foodstuff, known to have been burned in the body, by its caloric value. This accurate correspondence of the *direct* and *indirect* methods of calorimetry has both a practical and a theoretic interest. It shows, for one thing, that there can be no energy either created or lost in the animal body that is not accounted for by the oxidation of the foodstuffs. It shows that no energy can be absorbed from the outside or new forms of energy created in the animal body. It proves the law of the conservation of energy for the animal mechanism.

And now with regard to the application of these extremely important facts in the science of *dietetics*. We are in a position to determine with scientific accuracy just exactly *how much food should be taken under varying conditions of bodily activity*. In a general way, we know that the amount of food that is required is proportional to the nature and amount of bodily exercise that is being performed at the time, and that, if the food supply is inadequate, the work before long will fall off, not only in quantity, but in quality as well. "Horses (and men) work best when they are well fed, and feed best when they are well worked," is an old adage and one the truth of which cannot be overestimated in the consideration of all questions of dietary requirements. An ill-fed beggar will rather suffer from the pain and misery of starvation than attempt to perform the piece of work that the well-meaning housewife bargains should be done before she gives him the meal. The spirit may be willing but the flesh weak. If he could be trusted, he should be fed first and worked afterwards. Besides the amount of work, two other factors are well known to influence the demand for food, namely, growth and climate. A young growing boy will often demand as much if not more food than would appear, from a comparison of his body weight with that of his seniors, to be his proper share, and, other things being equal, it is well known that we are inclined to partake much more heartily of food during the cold days of winter than during the sultry days of July and August.

That we know these facts, in a general way, indicates that the first steps in the exact determination of dietetic requirements must be to find out how much energy the body expends under varying conditions of activity, etc. It must be plain from what has already been said that this may be done by having the person live for some time in a respiration calorimeter, so that we may measure the caloric output by both the direct and the indirect methods, the results of the one serving as a check on those of the other. To the conclusions drawn from results of observations made under such artificial and unusual conditions of living, the objection might, quite justly, be raised that they need not apply to persons going about their ordinary routine of life. To meet this objection another method, which we may call the *statistical*, is available. This consists in taking the average diet of a large number of individuals and comparing its caloric value with the average amount and type of work that they are meanwhile called upon to perform. This can be done in cases where the diet is accurately known, as in public institutions, the army, the navy, etc. The total food supplied is then divided

by the number of individuals, this giving the *per capita* consumption. Obviously some get more than others, but when a sufficient number of individuals is included, such errors become eliminated by the law of averages. The close agreement between the results secured by these two quite different methods is a guarantee of the reliability of either.

Before proceeding to consider the results in greater detail, it is plain that, in order to make it possible to compare the energy output of individuals of different sizes, the results, that is, the caloric output, must be determined for some standard size of body. If such a standard were not used but we merely quoted the results as so many calories given out during each hour or day, we should, of course, find that a small child gave out far less energy than a large full grown man; we should fail entirely to bring out the fact, which is of great significance, that relatively to the size of their bodies the child gives out considerably more energy than the adult. The standard usually employed is that of body weight, the kilogram ( $2\frac{1}{4}$  lb.) being the unit. During recent years, however, it has been claimed that a more accurate basis of comparison is the unit of body surface, the square meter; but with the reasons for this change of standards and the controversial matters which have been raised in connection with them, we need not concern ourselves; for the purposes of a scientific study of the principles which govern dietary requirements, the body-weight standard is the most suitable to adopt at present.

Let us then consider the caloric output of a healthy man of average weight (70 kg.). In the first place, it must be measured while he is at perfect bodily rest, lying quietly in bed, and at such an interval of time after taking food that the digestive organs are inactive. This is done by having him sleep in a bed placed in the respiration calorimeter and measuring the caloric output the first thing in the morning when he awakes. Under such conditions it has been found that 1 C. per kg. per hour, or  $1 \times 70 \times 24 = 1680$  C. per diem, is produced. At first sight this result would seem to indicate that food containing an amount of protein, fat and carbohydrate capable of yielding 1680 C. would meet the daily requirements, but such is not the case. More must be given to allow for the fact that the physiologic processes involved in the assimilation of food by the tissues, quite apart from anything else, causes some heat to be dissipated from the body. This property of food has been clumsily called its *specific dynamic action*, and it varies according to the nature of the foods, being largest in the case of protein and smallest in the case of carbohydrate. As a rough estimate, it is usually considered that the average daily diet has a specific dynamic value amounting to ten per cent of the resting caloric output, giving us therefore, for a resting man living on an average diet, a daily caloric output of  $1680 + 186 = 1848$  C.

The further expenditure of calories depends entirely on the amount of muscular work done, and much interesting information has already been collected, showing just how many calories are set free in the performance of different types of work. It is significant that, with their far-sighted appreciation of the value of science in the welfare of the state, the German authorities should some years ago have appropriated considerable sums of money for just such investigations, and that the data should have served as one of their main guides in the apportionment of food to the people. The government, as soon as it saw



the possibility of a shortage, placed the control of food in the hands of scientific food experts without permitting such a vital question to be trifled with by legislators whose knowledge of food values is as shallow as their knowledge of how to play obstructive politics is profound. The delay that has attended the passage through Congress of the bill for food control is all the more provoking when we consider that in our country, and ready to serve voluntarily, are men who have convincingly shown themselves to be second to none in the world, not only in the scientific knowledge which must guide, but also in the executive ability which must administer, an efficient food control.

It has been found that if the person instead of lying in bed be made to sit still in a chair in the calorimeter, his caloric output increases by about 8 per cent, and if besides this he be made to do such work as writing at a desk, it will increase by nearly 30 per cent. A little simple arithmetic thus shows us that for such work the caloric expenditure per hour while the man is doing such work will increase from 77, which we saw to be that of basal heat production and the specific dynamic action of the food, to 97 C.; and if he does such work for sixteen hours and sleeps eight hours, this gives  $8 \times 77 = 616 + 16 \times 97 = 1552 = 2168$  C. A great increase in caloric output is created by walking, even on the level, and it has been quite clearly shown that the difference between the value which we have arrived at, viz., 2170 C., and the actual caloric expenditure of 2500 C., which from statistical studies is known to be expended by individuals doing a light day's work, can readily be accounted for by the walking incidental to moving from place to place in the daily routine.

Coming now to the results of the statistical method, the reliability of this method is testified to by the remarkable correspondence in the caloric values of the food consumed by farmers in widely different communities:

	Calories
Farmers in Connecticut,	3,410
"    "    Vermont,	3,635
"    "    New York,	3,785
"    "    Mexico,	3,435
"    "    Italy,	3,565
"    "    Finland,	3,474
Average,	3,551

(Lusk: The Fundamental Basis of Nutrition)

The average inhabitant of various cities:

London,	2,665
Paris,	2,903
Munich,	3,014
Königsberg,	2,394

(Rubner)

Individuals in different callings:

Farmers' families (U. S. A.),	3,560
Mechanics' " " "	3,605
Professional men's families (U. S. A.),	3,530
Army (U. S. A.),	3,851
Navy " "	4,998

(Atwater)

In general it is usually computed that a man weighing 70 kg. requires in calories:

2,500 for a sedentary life.

3,000 for light muscular work.

3,500 for medium muscular work.

4,000 and upwards for very heavy toil.

(McKillop)

These figures apply to the average man, but in calculating the caloric requirements of a family or a community allowance must be made for the lesser requirements of women and children. Several dieticians have compiled tables showing how many calories are expended according to age and sex, and the German authorities have recently taken these figures from them and calculated a generalized mean, which shows in comparison with men the percentage that should be allowed for women and children. The figures are as follows:

Man,	100
Woman,	83
Boy over 16,	92
Boy, 14-16,	81
Girl, 14-16,	74
Child, 10-13,	64
Child, 6-9,	49
Child, 2-5,	36
Child, under 2,	23

(McKillop)

In calculating the caloric requirement of the population as a whole, the necessity of making allowance for the varying needs of men, women and children would obviously make the calculations far too complicated for practical purposes. It is necessary to have a factor by which we may multiply the total population in order to determine its *man value*. This factor is based on the relative proportion of men to women and children, and it amounts very nearly to 0.75; i. e., three quarters of the total population gives "the man value." Knowing the total population, say, of a city, we must therefore multiply this by 0.75 in order to ascertain for how many men doing moderate muscular work (3000 C.) food has to be provided.

Although the first step in estimating the dietary requirements of a family or community is thus to ascertain how many calories are expended by each individual and then to find suitable foodstuffs that will supply this amount, it must not be imagined that we have thereby fulfilled all the conditions to be considered in drawing up a correct dietary. There are many other factors to consider, and these, for simplicity's sake, we may divide into two groups: first, those pertaining to the chemical nature of the foodstuff, and secondly, those pertaining to its palatability, digestibility, and availability.

To appreciate the importance of the *chemical nature of foods*, it will be well to return to the analogy of the animal body with a steam engine, not because we shall find that the analogy becomes any closer, but, because it so entirely breaks down in one important particular that it becomes of value on this very account. The fuel of the engine is fuel alone; it is used for no other purpose, whereas the food of an animal, besides being fuel, is also used to repair the tis-

sues of the body which have become broken down on account of the constant wear and tear to which they are subjected in carrying on the processes of life. The next step is, therefore, to find out the relative importance of the foodstuffs in supplying material for the reparative processes in the tissues. Biochemical investigation has shown that these are composed of the same classes of chemical substances as the foods—proteins, fats, carbohydrates, salts, and water—and that proteins occupy the most important position, since the greater part of the living tissue is composed of them; namely, the cell and its nucleus. Some fats or fat-like substances are also associated with protein in the construction of this vital tissue machinery, but by far the greatest bulk of the fat found present in the body merely represents storage fuel deposited, not in, but between the really active or vital tissues. Some carbohydrate is also probably used to construct the machinery, but by far the greater proportion is used for fuel purposes; indeed, carbohydrate is the most readily available of all the foodstuffs for purposes of producing energy. There is no large store of carbohydrate in the body, because it is quickly consumed, whereas fat may be stored away for some considerable time before it is ultimately used as fuel material. Standing distinctly apart from the others in dietetic importance, therefore, is protein. With this foodstuff alone, many animals can exist, although they may not thrive, whereas with fats and carbohydrates as the sole foodstuffs, life is impossible. Protein in the diet is a *sine qua non* of life, because it is more than a mere fuel: it is also the essential building material for the worn out tissues.

This unique position of proteins has, in a general way, been appreciated for many years, but, apart from the fact that, of all foodstuffs, proteins alone contain nitrogen, so little was known concerning their chemical structure that, with the exception of gelatin, all proteins were thought to be of much the same dietetic value. Thanks to the advancement of biochemical knowledge, it is now known that this view is very far from being correct, for proteins differ in their chemical structure and in their dietetic value. The differences are dependent upon the nature and proportions of the various groups of smaller molecules of which the highly complex and very large molecule of protein itself is composed. To make this clear, let us imagine the protein molecule as a completed building with its stone and lime, its woodwork and plumbing, its plaster, and so on. It is composed of a great variety of building materials; but in a row of buildings no two need be exactly alike (although the same materials in general are used in their construction); some of them may have no stonework, others no plumbing, and even in those which use all of the available materials, the relative quantities used will vary considerably. So with protein: it is built up of innumerable building materials belonging to the class of chemical substances known as amino acids, that is, organic acids whose acidity is practically neutralized by the inclusion in the molecule of an ammonia residue, called the amino group. There is a great variety of such amino acids, some comparatively simple and others highly complex, since they contain, besides the organic and amino group, other chemical groups that are often of highly intricate structure.

To know the name and structure of each of these protein building materials, or amino acids, is not necessary for our purpose here, but there are two or three that we must at least mention in order to be, later on, in a position to

understand why certain proteins should be more valuable than others as food-stuffs. These are lysin, containing two ammonia groups; tyrosin and tryptophan, containing a so-called aromatic group; and cystin, containing sulphur. The protein of muscle, for example, is not composed of exactly the same variety and proportion of amino acids as that of egg white. Even the proteins of the same tissues of different animals may not be exactly the same in their amino-acid consistence. It is evident, then, that if, on account of wear and tear, the tissues should require certain amino acids with which to reconstruct their protein, the supply can be insured only provided the food contains proteins yielding these particular amino acids. In the process of digestion the proteins become broken down into the amino acids, which are then absorbed into the blood. The most perfect protein food would thus be one containing all of the amino acids found present in the proteins of all the tissues, for then each tissue could select from the blood the exact amount of each of the amino acids it required, and what one tissue did not require the others might make use of. In this manner all of the amino acids of a protein foodstuff might theoretically be used as building material for worn out tissue protein; but such a perfect adjustment between supply and demand does not actually occur, there being always a surplus of some amino acids, just as there would surely be some building material unused from a wagon, initially filled with every variety, after it had supplied to each one of a row of houses the materials required for repair purposes. This rejected building material has to be got rid of from the body, and this is accomplished by the amino acid being split up into two parts, of which the one is burned to yield energy, and the other, consisting of ammonia, is excreted in the urine as urea.

The proteins that contain all of the essential amino acids, though in varying proportions, are those of animal origin, such as the casein of milk and the albumin and globulin of blood, eggs, and muscle. Certain vegetable proteins, such as are present in part at least in the soy bean, hemp seed, Brazil nut, maize, and wheat (glutenin), also contain all of the necessary amino acids, though not in such suitable proportions as in proteins of animal origin. These may be designated as vegetable proteins of the first quality. Other vegetable proteins, such as those of beans, peas (legumin), part of the protein of maize (zein) and wheat (gliadin), etc., on the other hand, are wanting in one or more essential amino acids and may be designated as of second quality.

These facts have been ascertained by actual chemical analysis of the proteins, and their relationship to the building up of tissue proteins has been demonstrated by observing the rate at which young animals grow when fed on different proteins. During growth it is plain that the building-up process in the tissues is occurring in exaggerated form, so that by observing the weights of the animals from day to day the rate of the process can be measured. It is no doubt the case that proteins which are inadequate for growth, will also be inadequate for the repair of broken-down tissues in the adult. By taking a piece of paper ruled in squares and placing the weights of the animals on the horizontal lines and the days of observation on the vertical, we obtain what is known as *the curve of growth*.

Many of the earliest observations were made on young rats and mice. To eliminate individual errors large numbers of the animals were used, all of them being fed on a uniform diet of carbohydrates, fats, and salts, to which

was then added the particular protein whose influence on growth it was desired to investigate. Large numbers of animals were used in each group, so as to eliminate individual peculiarities and accidental errors. Fed on the basal diet alone, the animals did not live for more than a few days. If protein of animal origin, such as the casein of milk or the albumin of milk or egg, or blood were added, however, the curve of growth was exactly like that of a normal animal. The proportion of protein that had to be given to attain normal growth varied considerably in different animals. In the case of white mice 25 per cent of the total calories had to be given as protein; rats required 15 per cent, and man seems to need only 7 per cent, this being the proportion in human milk on which alone the human infant thrives. With vegetable proteins, such as the glutenin of wheat and maize (Indian corn), which contain all the amino acids, normal growth could also be secured, but more of the protein had to be given than was the case with animal proteins, because some of the amino acids are not present in adequate amounts. With vegetable proteins in which certain amino acids were missing, however, the animals did not grow at all. Thus, with one of the proteins of maize known as zein, the curve of growth actually descended, showing that the animals must soon have died of starvation. Chemical analysis shows that two essential amino acids are wanting in zein, namely, lysin and tryptophan. By adding pure tryptophan along with the zein, a distinct improvement was noted in the curve; it no longer descended, but remained practically horizontal, indicating that now, although incapable of growing, the animals were being at least maintained. Evidently, then, proteins must contain tryptophan if they are to prevent starvation. If lysin, as well as tryptophan, were given along with the zein, the curve of growth became normal, that is, it became the same as that obtained when perfect proteins such as casein are fed. Lysin, therefore, must be an important amino acid for growth, and it is of great significance that there is a high percentage of lysin in all those proteins that are concerned in nature with the growth of young animals; thus, it is present in large amount in casein, lactalbumin and egg vitellin.

The condition of the animal that has been fed on inadequate proteins is of great interest. When the aromatic amino acid, tryptophan, which, as we have seen, is essential even for maintenance, was absent, the animal soon passed into a serious condition of malnutrition. Its fur became ruffled, its eyes inflamed, its feet cold, and it remained in a condition of torpor. By adding tryptophan to the diet, these symptoms immediately disappeared, and the animal became perfectly normal in every respect except that it failed to grow. It remained healthy but stunted. A most interesting question here presents itself, namely, has the power to grow become lost, or has it merely become suppressed by the absence of lysin? It is of great significance that growth merely becomes suppressed, for when the stunted animal was given a perfect protein, such as casein, it immediately began to grow with great rapidity, and soon attained the size of its now full-grown brothers and sisters.

Although it is particularly the vegetable proteins that are likely to be deficient in essential building stones, certain animal proteins, such as gelatin, are also lacking. Gelatin, like zein, contains no tryptophan, and like zein, it can not,

therefore, maintain life. It is not a true protein but it is a valuable adjunct to protein food, because it contains many useful amino acids.

It should be emphasized that in wheat and maize besides the imperfect proteins, gliadin and zein, there is also another protein that is of first quality, namely, glutenin. This is present in sufficient amount to make even strictly vegetarian diets perfectly safe, provided enough of either of these cereals is taken to allow for the fact that only a part of the protein is of first quality.

Now it will be asked, how are we to make certain that suitable variety of protein building stones is present in the diet? The answer is that there is little chance of inadequacy in this regard provided several varieties of protein food are given. "Provide sufficient calories and let the proteins take care of themselves," is a perfectly safe rule to work by, provided animal proteins are used. Real danger from protein starvation could arise only in the case of strict vegetarians who did not take a sufficiency of wheat or corn, for, although other vegetable proteins than glutenin do contain all of the essential amino acids, yet they may be deficient in this regard, and it would be decidedly risky to attempt to live on them alone. Strict vegetarians are, therefore, liable to run the risk of partial starvation. One of the most valuable of proteins is probably casein of milk; another vitellin of egg yolk. A glass or two of milk with an egg, along with vegetable food, makes the diet a safe one, provided always, of course, that the caloric requirements are met, and that no excessive wear and tear of the tissues is going on.

But it is probable that such a diet is inferior to one containing a proper, but not excessive, amount of animal proteins. It has been found that the smallest amount of protein required to maintain nutritional equilibrium is secured by taking flesh food, along with abundance of carbohydrate and fat, because obviously this, in its amino acid make-up, comes closest to that of the animal's tissues.

These considerations lead to the question: To what extent may the proportion of protein in the diet be reduced with safety? It is evident that there must be a minimum below which every one of the necessary building materials of protein would not be supplied in adequate amount to reconstruct the worn-out tissue protein.

The extent to which the protein content of the diet of man can be lowered with safety depends on several factors, of which the most important are: first, the nature of the protein; secondly, the number of nonprotein calories; and thirdly, the extent of tissue activity. Where so many factors must be taken into consideration, the only method by which the actual minimum can be determined consists in what may be called "cut and try" experiments. Of the many investigations of such a nature, probably the best one is that recently published from the Nutrition Laboratory of Copenhagen. The subject, an intelligent laboratory servant, lived a perfectly normal and active life for a period of five months on a diet of potatoes cooked with margarine and a little onion, and containing 4000 C., with a total protein content of 29 grams. During another period he did outdoor work as a mason and laborer, and took 5000 C. daily, and 35 grams protein. Many other experiments of a similar nature make it certain that man can lead a normal existence and remain in good health

on very much less protein than the 100 grams which statistical studies show to be the amount which he actually takes. This discrepancy between the amount which experiment demonstrates to be adequate and that which habit and custom demand, raises the question as to whether, after all, our instincts may not have erred and so made us unnecessarily extravagant in our protein intake. It has been suggested that such protein extravagances, will in various ways, have a deleterious effect on the organism; thus, that the excretory organs, such as the kidneys, will be overtaxed in eliminating the unused amino acids, that the constant presence of the bodies in excess in the blood will cause degeneration and sluggish metabolism, and that the excess protein in the intestine will lead to the production of ptomaines, whose subsequent absorption into the blood will cause toxic symptoms.

Important support to such views appeared to be supplied some dozen years ago by Chittenden, who was able to show that he himself and many other persons doing different kinds of work could be supported on daily amounts of protein that were not more than from one-third to one-half of the amount usually taken. Not only so, but it was averred that distinct improvement was experienced in the general sense of well-being and of mental efficiency as a result of the lesser protein consumption.

Taking these results as a whole, it is quite clear that man can get along under ordinary conditions with much less protein than he ordinarily takes. This really proves nothing, for the question is not *can* he, but *should* he so deprive himself. Are instincts and customs wrong, and is Chittenden right? That is the question. To answer it many studies have been made of the condition of peoples who for economic or other reasons are compelled to live on less protein than the average. Are these people healthier, less prone to infections and degenerative diseases, and more efficient mentally than others? In such studies great care must be exercised to see that conditions other than diet, such as climate, exercise, etc., are properly controlled. It would not, for example, be fair to compare the mental and bodily condition of peoples living in the tropics and who take comparatively little protein, with those living in temperate zones, who consume much more. After discounting all of these other factors, it has been quite clearly shown that, when the protein allowance is *materially reduced*, the people as a whole are less robust, mentally inferior and, instead of being less prone to the very diseases which are usually supposed to be due to overloading of the organism with useless excretory products, they are more liable to suffer from them. That a considerable reduction in protein weakens the defense of the organism against infection is probably due to the fact that the fluids of the body normally contain a great variety of so-called antibodies, that is, of highly complex substances that are largely protein in nature. When bacteria, or the poisons produced by them, enter the body, they are met by one or more of these defense substances and destroyed or neutralized. Now it is clear that there should always be a surplus of protein building materials from which these antibodies may be constructed. Such an excess will constitute a "factor of safety" against disease. And there are factors of safety of another nature to be produced, two of which we are in a position to appreciate. In the first place, there must always be an adequate supply of

tryptophan, of lysin, and of cystin, not only to meet the bare necessities of the protein constructive processes that go on under normal conditions, but also to make good the larger amount of protein wear and tear that greater degrees of tissue activity will entail. Although moderate muscular exercise does not appear to cause any immediate consumption of protein (carbohydrate and, later, fat being the fuel material used to produce it), yet it does throw a greater strain on the tissues, a greater wear and tear of the machinery, and hence a demand for more protein building material. In the second place, there are certain of the internal secretions of the body, such as epinephrin (adrenalin), which are essential for life, and as crude materials for the manufacture of which certain amino acids are essential. Tyrosin is one of these, and since, as we have seen, proteins differ from one another quite considerably in the amount of this amino acid which they contain, it is advisable to provide an excess so that an adequate supply of tyrosin may always be available.

The answer to one of the most important practical questions in dietetics, namely, "What proportion of protein should the diet contain?" depends on these scientific principles. The source of the protein is the important thing. With animal protein there is no doubt that we could get along with perfect safety by taking daily not more than 50 to 60 grams, which is about half of what we actually take. If the protein be of vegetable origin and of the first quality, such as is contained in wheat and maize preparations, more should be taken, because of the fact that the protein in these cereals is only half of the first quality. When vegetable proteins of the second quality, such as those of peas, beans, lentils, etc., are alone available, much larger amounts are necessary. Such proteins are inadequate in the case of growing children at least, and even in adults it is undoubtedly advisable that other proteins should supplement them.

To insure safety, therefore, it is almost imperative that the diet should contain *proteins of various sources*. If for economic reasons the main source must be proteins of vegetable origin, then some animal protein, such as is contained in milk or meat or eggs, should be added to at least one of the daily meals. Thus, when peas and beans are mainly depended on for the protein supply, they should be taken either with milk or one of its preparations, or with a thick gravy or sauce made from meat and containing the finely minced meat. This must not be strained off, for if it is, the sauce will contain only the meat extractives but not any of the protein, for these are coagulated by the boiling water. Meat extract, in other words, contains no proteins; it is not a food but merely a condiment of no greater dietetic value than tea or coffee.

The question has been asked, "What should we take in place of meat if one or two meatless days have to be introduced in order to conserve the food supply?" The answer is that milk and eggs will completely make good the deficiency, or if these also be unavailable, then the taking of a more liberal supply of wheat or maize preparations will be satisfactory. Protein deficiency for one or two days a week could, however, scarcely entail any risk to health provided the usual allowance of animal protein or of first quality vegetable protein be allowed on the other days. The value of potato protein should be remembered in this connection. In any case the attempt should always be made



to give a variety of proteins. That the animal economy prefers, if it does not demand, such a mixture even if the best varieties of protein is indicated by the fact that milk, the perfect food for early growth and development, contains two such proteins—casein and albumin.

Regarding the relative quantities of fats and carbohydrates, the usually accepted figures are: fat, 80 grams (3 oz.); carbohydrates, 400 grams (14 oz.); that is, a ratio of 1 to 5.

Even when the calories and the protein are correct, the diet may be inefficient because of absence of minute quantities of peculiar substances of unknown composition. These have been called *vitamines*, but this is a most unfortunate name, since an amine is a well characterized chemical substance, whereas these "accessory food factors," as they are better called, are not. Failure of nutrition due to the absence of accessory food factors is really no recent discovery. It was known to sailors in the bygone days of sailing vessels that despite a liberal allowance of well-mixed preserved food, a long voyage was almost certain to lead to the development of ill health, despondency, and incapacity for work, or perhaps of the disease, scurvy, itself. The discovery was, however, made by the famous Captian Cook that this unhealthy condition of his sailors could be relieved by compelling them whenever possible to go ashore and eat of the fresh foods, either animal or vegetable, that might be available. It was perfectly clear that such foods contained something of great benefit to health that was lacking in the ship's galley. The giving of orange or lemon juice in certain cases of malnutrition in children has also been known for some time in medical practice, but the impetus to a more searching investigation of the nature of these unknown accessory food factors was given by the discovery that the curious disease called beriberi, often observed in certain tropical countries, was associated with the taking of polished rice in place of the less popular grain still having some of the husk attached. This observation led to a systematic investigation of the association between this and the analogous disease which develops in pigeons when these birds are fed exclusively on polished rice. It is found that the addition to the polished rice of an alcoholic extract of the husks very promptly removed the symptoms, and that other things like yeast had a similar effect. Several investigators attempted to isolate this *vitamine*, as they called it, in pure condition, and thus determine its exact chemical composition, but with little success. Among the most careful of these investigations are those of McCollum, who has come to the conclusion that there are at least two accessory factors concerned, one of them soluble in fat and present in adequate amount in butter and other animal fats, but not in vegetable oils, and the other soluble in water and present in wheat, vegetables, fruits, etc. Milk contains both of these factors, so that its inclusion in a diet is a safeguard, not only against inadequacy in suitable protein, but also against the absence of accessory food factors. There is little danger of the diet being inadequate with regard to food factors if it contain some fruits or green vegetables or unheated fresh milk. The food factors are destroyed by prolonged cooking.

*Digestibility and Palatability.*—No matter how perfect in calories, protein,

and accessory food factors a diet may be, it will fall short of being really adequate if it is not properly assimilated. It is the function of the digestive apparatus to break up the highly complex molecules of protein, fat, and carbohydrate sufficiently to permit them to pass through the lining membrane of the intestine into the blood and lymph. This disintegration is effected by the digestive ferments which are contained in the digestive juices. Of these, saliva, the gastric juice, and the pancreatic juice are best known. In the cases of protein and carbohydrate, the activities of the ferments are interdependent, in the sense that the foodstuff must be acted on by the ferments in a definite order. One ferment prepares the foodstuff for the action of the next. Without this preliminary treatment the second ferment can not properly unfold its action. The conditions are like those existing in a factory where the products of one department are further worked up in another, which then hands on its product to a third and so on.

These facts indicate that for efficient digestion it is essential that the *initial* digestive juice be secreted in proper amount and at the proper time. The first digestive ferment which acts on protein is the pepsin of gastric juice. A fundamental question in dietetics is: On what does the secretion of this juice depend? The answer is: On the gratification of appetite. No one, judging from his own experience, will probably deny the correctness of this answer, for we all know that unappetizing food is likely to be followed by a sense of gastric discomfort, if not by symptoms of indigestion. Are there, however, any scientific observations from which the true value of this factor in the initiation of the digestive process can be appraised? Thanks to the brilliant work of the great Russian physiologist, Pavlov, we have such information. Pavlov's experiments were made on dogs, but the results have been shown, particularly by Carlson, to be very similar in the case of man. The observations were made on animals in which an artificial opening or fistula had been made into the stomach. Through this fistula the secretion of gastric juice could be observed, and it was found that very shortly after taking some savory food by the mouth, a copious secretion of gastric juice was set up, and that this occurred even although the food after being swallowed was prevented from entering the stomach, by making a fistula in the esophagus. The experiment is called "sham feeding." Not only this, but a hungry animal would secrete the gastric juice even although the food was not actually placed in the mouth but merely offered to it. The anticipation of an appetizing meal, as well as the gratification of receiving it, can set up the flow. It is called the *psychic* or *appetite* juice. If the animal were not hungry or had no appetite for the particular food, no juice was observed to flow.

The pepsin in this psychic juice sets the ball of protein digestion rolling. Once started, this process goes on automatically, because the digestive products produced by the appetite juice have the power of directly stimulating the gastric glands to further activity, and when the food has been digested to a somewhat further stage, the stomach delivers its contents, in small quantities at a time, into the beginning of the intestine, where by again acting directly on the lining membrane it excites the flow of pancreatic juice, a ferment of which, namely, *trypsin*, carries the digestion to still another stage, until finally the

protein molecule is sufficiently broken up to be attacked by the ferment *crepsin* present in the intestinal juice and intestinal mucosa. The whole process, therefore, depends for its proper accomplishment on the appetite juice. It is like a fire: the psychic juice is the kindling material; when it is ignited, the combustion goes on automatically, one stage leading to the next. Some substances, such as the so-called extractives of meat, act like partially digested protein in directly stimulating the secretion of gastric juice.

We have seen that practical dietetics depends on several factors, the exact relative importance of which can not perhaps in every case be gauged, but preparation of the food so as to make it appetizing, must undoubtedly rank high. The importance of *good cooking* will now be apparent. It is the act of making food appetizing and, therefore, digestible. It is really the first stage in digestion, the stage that we can control and one therefore to which much attention must be given, especially when it becomes necessary to make attractive articles of diet ordinarily considered common and cheap. Most people can cook a beef-steak or a lamb chop so as to make it reasonably appetizing, but few can take the cheaper cuts of meat and convert them into cooked dishes that are as popular and attractive. There are still fewer who can take the left-overs and trimmings and convert them in the same way. This is the real art of cooking, and too much encouragement can not be given to the effort which our cooking experts are making to show people how these things can be done. The waste of good food in a large city is appalling. An army could live off our garbage cans. I need not dwell on this most important phase of the food conservation problem. I would only add that every housewife who desires to do her "bit" in the present emergency can do so in no way better than by learning to use *all* the odds and ends of the kitchen in such a way that they can be offered as appetizing food to her household. It is worse than useless to dish things up unattractively, for under such circumstances food becomes poison.

Cooking has other advantages than making the food appetizing. The heat loosens the muscle fibers of the meat so that it is more readily masticated; it destroys microorganisms and parasites in the meat; it destroys antibodies which might interfere with the action of the digestive ferments. Thus, raw white of egg is not digested in the stomach because it contains one of the antibodies which prevent the pepsin from acting on it. Boiled egg white, if properly chewed, is digested, and whipping the egg white into foam partly destroys the inhibiting substance.

Before concluding, something should be said about the *laxative qualities of food*, for it is often in this particular alone that one food is more satisfactory than another. A diet of meat, milk, eggs, and white bread is apt to be unphysiologic because there is nothing in it to serve as what has been called intestinal ballast, that is, a material which will keep the intestines sufficiently filled to stimulate their muscular movements. This ballast is best furnished in the shape of cellulose, the most important constituent of green food. Peas, beans, cabbage, salad, and many fruits, especially apples, should always occupy a place in the daily menu. Another valuable food yielding this ballast is the outer grain of wheat, oats, etc. So much must not be taken as to produce a constant intestinal irritation, and each person must determine for himself where

this limit lies. The difference between various breads is almost entirely in the degree to which they supply ballast.

#### APPENDIX.

It will be remarked that nothing is said in the foregoing article concerning the methods by which a given diet can be composed so as to supply the required number of calories. This is a detail which could be adequately discussed only by reference to extensive diet tables, the publication of which is unnecessary here. It may help if we give some rough and ready rules by which such tables may be satisfactorily used. As a type of diet table take the following:

FOOD	PROTEIN PER CENT	FAT PER CENT	CARBOHYDRATE PER CENT	CALORIES PER LB.
Average of beef, veal, and mutton	14.5	16.1	...	913
Pork	12	29.8	...	1477
Bacon	9.5	59.4	...	2685
Fish (general average)	10.9	2.4	...	295
Eggs (2 oz. in shell)	11.9	9.3	...	613
Milk (whole)	3.3	4	5	322
Cream (average)	2.5	18.5	4.5	908
Butter	10.	8.3	...	3510
Cheese	25.2	33.7	2.4	1950
Bread (average)	9.2	1.3	53.1	1215
Rice	7.4	0.4	79.2	1620
Legumes (dried average)	2.4	1.7-2.3	54	1500
Potatoes	1.8	0.1	14.7	310
Green vegetables	1.4	0.2	4.8	145
Fruit (average)	0.4	0.5	8.	180

(McKillop)

Having decided how many calories a day is required according to the principles laid down on page 747, proceed to weigh out each of the articles of food that it has been customary for the person or persons to take. If the calories do not correspond to the required number, add or subtract a proper amount of one or more foodstuffs, using the figures in the fourth column of the table for this purpose. Having adjusted the food allowance according to calories, proceed to see whether there is sufficient protein according to column 1 and the principles explained on page 756. The simplest way to do this is, first, to multiply the number of ounces of each foodstuff used by 28.4 (grams to an ounce), and then by the percentage figure given in the first column. The product divided by 100 gives the grams of protein. Finally calculate by the same method the grams of carbohydrate and fat, and see that they bear the ratio to each other of about 4 to 1.

Finally, it should be remembered that the above requirements refer to foodstuffs actually digested. In the case of protein, 10 per cent is usually subtracted from the crude protein in arriving at this figure. For fats and carbohydrates the figure is quite variable. The figures in the above table are also for raw materials. Where there is evident loss in cooking, proper allowance must, of course, be made.

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# CHEESE POISONING A TOXICOGENIC BACILLUS ISOLATED FROM CHEESE\*

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## HISTORICAL INTRODUCTION.

CHEESE poisoning, according to Husemann,<sup>1</sup> was already known in North Germany in the sixteenth century. Christison,<sup>2</sup> in his "Treatise on Poisons," stated: "During the third quarter of the last century (eighteenth) this kind of poisoning was so common that several of the German states investigated the subject, and legislative enactments were passed in consequence. For a long time the prevalent belief was that the cheese acquired an impregnation from copper vessels used in the dairies, and accordingly the Austrian, Wirtemberg, and Ratesberg states prohibited the use of copper for such purposes. This opinion was proved by chemical analysis to be untenable." Morgagni<sup>3</sup> mentions arsenic as the cause of cheese poisoning. Later investigators ascribed the poisonous properties to lead, zinc, and mercury.

Early in the nineteenth century the experimental method of inquiry was begun; and although it was not until the latter half of the century that the cause was definitely established, the work done in the interim was of value from the negative point of view. Hunnefeld,<sup>4</sup> in 1827, analyzed poisonous cheese and experimented with extracts upon the lower animals. He considered the active poisoning agents to be sebacic and caseic acids. "About the same time, Serturner, making analyses of poisonous cheese for Westrumb, also traced the poisonous principles, as he supposed, to these fatty acids."<sup>5</sup> This view was adhered to by succeeding investigators until 1852, when Schlossberger proved conclusively that the fatty acids, in a pure state, were devoid of poisonous properties.

In the years 1883 and 1884 there were reported to the Michigan State Board of Health about three hundred cases of cheese poisoning. Vaughan,<sup>6</sup> working on the cheese sent to him for analysis, was able to isolate a ptomaine, called by him tyrotoxicon, which he considered the active agent in the poisonous cheese. His work was corroborated by Wallace<sup>7</sup> in 1887, by Wesener and Rossman<sup>8</sup> in 1898, and by Newman<sup>9</sup> in 1902. Lepierre,<sup>10</sup> in 1894, isolated a base having the formula  $C_{16}H_{24}N_2O_4$  from poisonous cheese. Dokkum,<sup>11</sup> in 1895, isolated a base, a currare-like poison, from putrefactive cheese, which he called tyroxin. Recently (1910) Spica<sup>12</sup> obtained a toxic extract, of unknown chemical composition, from poisonous cheese.

From the work of these men it is evident that they considered cheese poisoning an intoxication. In classifying the various bases obtained from poisonous cheese with the ptomaines, they moreover admitted the poisoning to be primarily due to bacterial activity. For a ptomaine, according to Vaughan and Novy,<sup>13</sup> "may be defined as an organic chemical compound, basic in character, and formed by the action of bacteria on nitrogenous matter." The question

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then arose: "Can pathogenic or toxicogenic bacteria be shown to be present in poisonous cheese?"

Bacterial analysis of poisonous cheese had already been undertaken by Sternberg, at the request of the Michigan State Board of Health in 1884. In his report<sup>14</sup> to that body he stated that he found "micrococci in the fluid of the cavities of the cheese." Subcutaneous injections of the pure cultures into rabbits were without effect. He concluded: "It seems not improbable that the poisonous principle is a ptomaine developed in the cheese as a result of the vital activity of the above mentioned micrococcus, or of some other microorganism which had preceded it, and had perhaps been killed by its own poisonous products."

Novy,<sup>15</sup> in 1890, isolated an organism from poisonous cheese which, when inoculated in milk and kept in the incubator, was fatal to cats. Vaughan and Perkins,<sup>16</sup> in 1895, isolated a toxicogenic bacillus from cheese which had proved poisonous to twelve persons. Their bacillus belonged to the colon group. Holst,<sup>17</sup> a year later, described a colon-like bacillus which he had obtained from poisonous cheese. Fonteyne,<sup>18</sup> in 1908, obtained a Gram-positive, pathogenic bacillus from a sample of cheese which had caused a gastrointestinal epidemic at Meirelbeke among forty persons. Kühl,<sup>19</sup> in 1913, working with a cheese that had caused poisoning to about fourteen persons, isolated a pathogenic organism which he considered similar to the *Escherich lactis aerogenes* bacillus. In all these cases it was demonstrated by animal experimentation that the organism isolated caused the poisoning. Vaughan and Perkins were able to show that their organism formed a soluble poison, as sterile filtrates of their cultures, when injected intraperitoneally, into guinea pigs, caused death. In the other cases of cheese poisoning from bacterial activity no studies were made, or at least reported, upon the toxicogenic properties of the organism.

An outbreak of cheese poisoning among several members of a family in Kalamazoo, Michigan, offered the opportunity to investigate and study the cause of the poisoning. The results of this investigation are embodied in this report.

#### HISTORY OF THE CASES.

Mr. and Mrs. B. W. M. and family of four children, of Kalamazoo, Michigan, on June 20, 1916, were taken seriously ill several hours after having eaten supper, which had included American cheese. Dr. W. S. Tomkinson, who was called in, sent a sample of the cheese, of about 100 grams, and a history of the case to the Hygienic Laboratory of the University of Michigan. His report follows:

"Case 1. Mr. B. W. M., osteopath, ate supper, including limburger cheese, about 6 P.M. At 12 P.M. purging; vomiting; face pale, muscles trembly; eyes dilated; pulse 110, irregular. Vomitus thin, reddish color with small chunks of cheese and undigested meat. 12:10 A.M. gave tablespoonful magnesium sulphate in  $\frac{3}{4}$  ii hot water. Purging profuse. O. K. at 3 A.M. with painful abdomen and hypothorax.

"Case 2. Mrs. B. W. M. Same as Case 1. Pulse 100, irregular; weak. 12 P.M., vomiting and straining, with flushed face. No purging. 12:10 gave magnesium sulphate tablespoonful to  $\frac{3}{4}$  ii hot water. Vomited immediately. Repeated dose; vomited in five minutes. Patient grew worse and rolled on floor with stomach cramps. Gave  $\frac{3}{4}$  i epsom salts to pint of hot water with immediate results and relief. O. K. 3 A.M.

"Case 3. Oldest boy, age 11 years. Was feeling better when I reached house at 12

midnight after purging and vomiting. Epsom salts cleaned out bowels thoroughly and gave relief.

"Case 4. Girl, age 8 years. At 6 P.M. ate heartily of cheese. 12 P.M., vomiting. Mother gave 2 5 castor oil. Girl quieted down and went to sleep. At 12:20 I woke her up and found she had a chill. Cold sweat. Pulse slow, irregular. She was weak and trembly. Eyes dilated, face pale, cyanotic. Said she felt all right, but was cold and wanted to be covered up and go to sleep again. Gave 5 ss magnesium sulphate which was thrown out of stomach with such force it struck opposite wall three feet away. Many small chunks of cheese in vomitus. Washed out stomach with warm water which returned tinged red. Repeated epsom salts 3 ss, water 3 ii, which started purging with relief. Patient O. K. and asleep at 2 A.M. Pulse weak, rapid, but regular. Color returned to face.

"Case 5. Boy, age 6 years. Asleep at midnight was awakened and took epsom salts 3 ss, hot water 3 ii, and immediately fell asleep again. Slept 20 minutes, after which purged out thoroughly. Pulse 60, slow, very irregular. Pupils dilated, face cyanotic, cold sweat. Not in as much pain as rest. After purging thoroughly—face flushed—went back to bed feeling comfortable. O. K. 3 A.M.

"Case 6. Paly boy, age 16 months. Ate sparingly. Gave magnesium sulphate 3 ss, hot water 3 ii, and put to bed. Slight cramps; free bowel movement. No vomiting. O. K. 3 A.M.

#### SYMPTOMS.

The symptoms as given by Tomkinson are those of known cases of cheese poisoning,—a fact that led him to regard his cases as those of tyrotoxismus. As a rule, the symptoms are of a severer character. Early observers<sup>20, 21, 22</sup> gave these symptoms as characteristic of cheese poisoning: chills, paleness of face, severe vomiting, diarrhea, marked pains in stomach and intestines, trembly limbs, thirst and dizziness. Pollius,<sup>23</sup> Wallace,<sup>7</sup> and Holst<sup>17</sup> mention vomiting of blood. Fonteyne<sup>18</sup> stated that some of his patients passed bloody stools. At times marked nervous disturbances may occur, as in a case cited by Vaughan,<sup>16</sup> where a patient became violently delirious. In the Michigan epidemic of 1883-1884<sup>24</sup> the symptoms produced by poisonous cheese were: dryness of the throat, nausea, vomiting, diarrhea, nervous prostration, headache, and sometimes double vision.

#### INCUBATION PERIOD.

Significant and important is the interval of time between the eating of the cheese and the appearance of the first symptoms of poisoning,—an interval which must be regarded, from the majority of cases, as an incubation period. This terminology would imply that cheese poisoning should be regarded not as an intoxication but rather as an infection. This is, indeed, the opinion of the present-day investigators.

The incubation period in the cases with which this report is concerned was, it may be remembered, six hours. It would be reasonable to assume that, were we dealing with an intoxication, the symptoms would have appeared much earlier. Instances are cited in the literature<sup>1, 20, 21</sup> where the interval of time between the eating and the appearance of the symptoms was from one to two hours. Westrumb<sup>2</sup> mentioned half an hour interval. In the Michigan epidemic of 1883-1884 some persons were taken ill from thirty minutes to four hours after eating. In such cases a preformed poison may be considered, like that of Vaughan's tyrotoxon. In all cases, however, where bacteria were isolated

from poisonous cheese, the interval was not less than five or six hours,—a period amply sufficient for bacterial activity.

#### DURATION OF SICKNESS.

Prompt treatment by the attending physician resulted in a rapid recovery by the Kalamazoo patients. The poisoning, however, to which these people were subjected, was not so marked as in many of the cases cited in the literature. To this can be attributed the short duration of their sickness. Instances are not lacking where days and even weeks elapsed before the recovery of the patients. Thus Grimm<sup>20</sup> stated the period to be three days; Pollius,<sup>23</sup> eight to twenty-four hours; Zenker,<sup>22</sup> twenty-four hours; Wallace,<sup>7</sup> three days; Ehrhardt,<sup>25</sup> "a long convalescence;" Newman,<sup>9</sup> forty-eight hours; Hughes and Healey,<sup>26</sup> four to eight days; Holst,<sup>17</sup> four days to three weeks; and Köhl,<sup>19</sup> ten days.

#### MORTALITY.

Deaths through cheese poisoning make up a very small percentage of the total number of cases. In the Michigan epidemic no fatalities were recorded. Lochte<sup>27</sup> tabulated eleven fatal cases from cheese poisoning up to 1903. Berg<sup>28</sup> mentions a fatal case occurring in 1908.

#### POSTMORTEM APPEARANCE.

Not all the fatal cases gave the same postmortem pictures. Hunnemann<sup>1</sup> found the stomach and intestines to be inflamed. Cameron,<sup>29</sup> quoting Holm's<sup>30</sup> postmortem findings, said "There was extravasated blood in the lungs, which were slightly congested; they decrepitated. The left ventricle of the heart contained liquid blood; the right auricle and two left cavities contained numerous soft and recent clots. The stomach and other abdominal viscera did not present any abnormal appearances which would account for death." Hughes and Healey,<sup>26</sup> in their report of an epidemic of gastroenteritis attributed to cheese poisoning (tyrotoxinon having been found in the cheese) at Aldershot, England, in 1899, gave the following postmortem appearances in three fatal cases: "Intense congestion; inflammation and edema of the mucous membrane of the stomach and small intestines, varying in degree and locality, but in one case involving the stomach and the whole of the small intestine. Peyer's patches were practically unaffected, but the solitary glands were congested and prominent. There was no ulceration or visible entozoa and the colon was unaffected. In one case the small intestine contained loose terra-cotta colored fecal material. The liver, which was enlarged (from 70 to 78 ounces) and bile-stained, showed signs of fatty degeneration. The gall bladder was full but not markedly distended. The spleen was normal. The kidneys were enlarged and intensely congested; the capsule was nonadherent. In one case the cortex of both kidneys contained small cysts filled with clear fluid. The skin and other tissues were deeply bile-stained. The lungs were congested posteriorly. The thoracic and abdominal lymphatics were not noticeably enlarged." Berg's case<sup>28</sup> presented a picture of cholera nostras. It seems, however, that in many cases marked anatomical changes do not occur.



## APPEARANCE OF THE CHEESE.

Tomkinson, the physician attending the M. family, sent a sample of the cheese and also of the meat eaten at that meal. The meat, evidently a pork chop, was found on bacterial examination, to contain only *Bacillus mesentericus vulgaris*, and hence was dismissed from consideration as the source of poisoning. The sample of cheese was of about 100 grams in weight, of the variety known as American (wrongly termed Limburger by Tomkinson), and of normal appearance and odor. This normal appearance corroborates the statements of many investigators that the cheeses causing poisoning were seemingly in excellent condition.

## EXPERIMENTAL WORK.

*Feeding Experiments.*—Experiments were conducted with a view of determining whether poisoning could be induced in laboratory animals by feeding them the suspected cheese.

TABLE I.  
FEEDING SUSPECTED CHEESE.

June 24, 1916.	Guinea pig No. 1, 220 gm., fed 10 gm. cheese.	No effects.
June 24, 1916.	Rats No. 1 and No. 2, both fed 5 gm. cheese.	No effects.
June 24, 1916.	Mice No. 1 and No. 2, both fed 5 gm. cheese.	No effects.

None of the animals fed showed the least symptoms of sickness. This was to be expected in view of similar findings by other authors. Nüchel,<sup>31</sup> in 1836, stated that a dog was unaffected eating cheese that had caused marked poisoning in a man. Pollius<sup>23</sup> claimed to have observed trembling in a mouse fed with poisonous cheese. Holm<sup>30</sup> obtained negative results in his feeding experiments. Sternberg<sup>14</sup> reported negative results by feeding rats and dogs. Vaughan said,<sup>32</sup> "If two samples, one of good, the other of poisonous cheese, were placed before a dog or cat, the animal would invariably select the good cheese; but if only poisonous cheese was offered, and the animal was hungry, it would partake freely. A cat was kept seven days and furnished only poisonous cheese and water. It ate freely of the cheese and manifested no untoward symptoms. After the seven days the animal was etherized and abdominal section was made. Nothing abnormal could be found." Holst<sup>17</sup> and Kühl<sup>19</sup> also obtained negative results in their feeding experiments.

## ALCOHOLIC EXTRACTS OF CHEESE.

After removing aseptically a portion of the suspected cheese for bacterial analysis the remainder, 65 grams, was chopped up finely and digested with 150 c.c. absolute alcohol for three days at room temperature. It was then filtered, the filtrate evaporated to dryness, taken up in 50 c.c. distilled water, heated on a water bath, and filtered into a weighed dish. On evaporation to dryness a residue of 1.634 gm. was obtained. This was then taken up in 50 c.c. distilled water, and portions of 5 and 10 c.c. each were injected, intravenously, into guinea pigs. The animals showed no symptoms of disturbance. The same procedure with the sample of the pork chop also gave negative results. It was concluded, therefore, that no alcohol-soluble substance caused the poisoning, and that the source might possibly be traced if a bacterial analysis were made.

## ISOLATION OF THE ORGANISM—TECHNIC.

With a sterilized knife a section of cheese was cut so that all surfaces were free from external contamination. This was then placed in a sterile test glass, physiological salt solution added, the cheese thoroughly macerated with a sterile rod, and the resulting suspension filtered through a sterile cotton filter. 1 c.c. portions of the filtered suspension were then inoculated into glucose gelatin, milk, and bouillon tubes, and the latter two incubated at 37° C. for 24 hours. A layer of sterile oil to about a quarter of an inch in depth was put over the glucose gelatin and these tubes incubated at room temperature. At the end of 24 hours the glucose gelatin showed marked gas formation, the milk was curdled, and the bouillon had a very heavy growth.

From these three different media, after plating on agar and fishing numerous colonies, an organism was isolated which, when grown in milk, proved pathogenic to mice. The same organism was isolated from glucose agar plates, grown anaerobically in Novy jars, in an atmosphere of hydrogen gas.

## MORPHOLOGY.

The organism is a facultative anaerobic bacillus, resembling in form and dimensions the *Bacillus colon*. The size of the bacillus varies from coccus-like forms to large rods, depending upon the kind and age of the media in which it is grown. As a rule its length is about three times its width. The organism grows singly and also in the diplo form. Thread formation has been infrequently observed. It does not form spores.

## MOTILITY.

Depending upon the media and the conditions of growth the bacillus may show active motility, sluggish motility, or even marked Brownian movement. Table II shows the influence of the kind of media upon the size and motility of the organism.

TABLE II.  
THE INFLUENCE OF MEDIA UPON SIZE AND MOTILITY.

AGE	MEDIUM	SIZE	MOTILITY
24 hours	Bouillon	Small rods; several large rods	Actively motile, large rods sluggishly motile
24 hours	Agar	Small rods	Actively motile
72 hours	Agar	Coccus-like bacilli	Motile
24 hours	Lactose broth	Slender rods, many large	Sluggishly motile, a few actively
24 hours	Dextrose broth	Plump rods, some very long	Marked Brownian movement; few slightly motile
24 hours	Saccharose broth	Small rods	Actively motile; many show no motility
24 hours	Milk	Small rods	Actively motile
72 hours	Milk	Small rods	Motile
24 hours	Uchinsky's medium	Fairly long rods	Many actively, some sluggishly motile
24 hours	Bile lactose	Small slender rods	Marked Brownian movement
Peritoneal fluid from guinea pig		Small plump rods	Few actively motile; most marked Brownian movement

## STAINING PROPERTIES.

The organism takes all stains readily. With Loeffler's methylene blue the ends of the bacillus stain a dark blue, while the inner portion resembles a vacuole. It is Gram-negative and not acid-fast. Smears made from the organs of an animal killed by the bacillus, when stained according to the Welch method, show the organisms to have capsule-like bodies around them. These, however, could never be verified as capsules.

## GROWTH ON GELATIN.

On gelatin plates the colonies are usually of two forms,—oval, characteristic of the deep colonies, and round, with irregular borders, characteristic of the surface colonies. Stabs show filiform growths, the growth being best at top. The gelatin is not liquefied.

## GROWTH ON AGAR.

The surface colonies on agar plates are round, with regular borders. Distinct concentric rings from two to three in number can be observed,—the inner ring being darker. The deep colonies are oval. When grown on glucose agar plates, large bubbles of gas, at times approaching 1.5 cm. in diameter, are formed. On agar slants the growth is abundant, filiform, and glistening. An odor is given off strongly resembling that of *B. coli* cultures. No pigment formation has been observed. On Drigalski-Conradi medium it forms pink colonies similar to *B. colon*. On Endo medium the colonies are red with the characteristic metallic caps of colon colonies.

## GROWTH ON POTATO.

The growth on potato is noncharacteristic. Depending upon the reaction of the potato the growth may be scanty or abundant—a slightly alkaline reaction favoring the latter. Where abundant, it is of a slimy consistency, raised, and at times spreading. After several days the potato becomes more or less browned.

## GROWTH IN NUTRIENT BROTH.

The bacillus grows readily and rapidly in nutrient broth, distinct clouding being visible in as short a period as three hours. A slight ring forms at the surface, but this may, at times, be lacking. No pellicle formation takes place. The clouding is strong at the end of twenty-four hours. On standing several days a flocculent sediment forms, with a slight clarification of the culture. The odor here again resembles that of a *B. colon* culture. In sugar broths growth is abundant, with formation of gas and acids.

## GROWTH IN MILK.

When first isolated the bacillus would curdle milk at the end of about four to five days. The curd in that case would be slimy. After passage through many animals (approximately 80), the organism curdled milk in forty-eight hours, and the curd was distinct from the whey. Gas bubbles could be observed at the surface of the culture. The acidity formed varied from 0.2 per cent to 0.3 per cent.

## GROWTH ON BLOOD SERUM.

On this medium the organism forms a moderate, slightly glistening, filiform growth.

## GROWTH IN USCHINSKY'S SOLUTION.

The solution was similar to that used by Vaughan and Perkins and was made up as follows:

Glycerine .....	40 parts
NaCl .....	1 part
CaCl <sub>2</sub> .....	0.1 part
MgSO <sub>4</sub> .....	0.4 part
K <sub>2</sub> HPO <sub>4</sub> .....	2.5 parts
Ammonium Lactate .....	6.0 parts
Asparagin .....	3.4 parts
Water .....	1000 parts

The bacillus grew copiously in this solution. In Fränkel's modification of Uschinsky's medium no growth was observed at twenty-four hours. A light growth was visible at forty-eight hours, which increased to a heavy growth at seventy-two hours. The laboratory strain of *B. colon*, and a strain isolated from feces also showed no visible growth in this solution at twenty-four hours, while both gave good growths in the Uschinsky medium for the same period. This is interesting in view of the fact that Vaughan and Perkins found their organism would not grow in Uschinsky's medium if the dipotassium hydrogen phosphate were replaced by disodium hydrogen phosphate. The latter salt, as can be seen from the following formula, was used in Fränkel's medium:

Asparagin .....	4.0 gm.
Ammonium Lactate .....	6.0 gm.
Na <sub>2</sub> HPO <sub>4</sub> .....	2.0 gm.
NaCl .....	5.0 gm.
Water .....	1000 c.c.

## TEMPERATURE REQUIREMENTS.

The optimum temperature is about 37° C. The organism grows also well at room temperature. At high temperatures, as at 45° to 50° it seems to lose its power of fermenting sugars. It grows scantily below 10° C. Cultures grown at 37° C. appear to be the most toxic.

## THERMAL DEATH POINT.

The thermal death point of the organism was determined by both the moist and dry heat methods, using silk thread, according to the technic employed in the Hygienic Laboratory of the University. The bacillus, as can be seen from the following tables, although withstanding moist heat at 50° C. for two hours is readily killed at 60° C. It is practically nonresistant to dry heat.

TABLE III.  
THERMAL DEATH POINT. MOIST HEAT.

Time in Minutes	½	1	2	3	5	10	15	30	45	60	120
50° C.							+	+	+	+	+
55° C.							+	+	-	-	-
60° C.		+		-	-	-	-	-			
70° C.		-		-	-	-	-				
100° C.	-	-	-	-	-						

TABLE IV.  
THERMAL DEATH POINT. DRY HEAT.

Time in Minutes	5	15	30	45	60
100° C.	+	-	-	-	-
120° C.	-	-	-	-	-
130° C.	-	-	-	-	-
150° C.	-	-	-	-	-

Controls for both moist and dry heat = +.

#### FERMENTATION.

Various sugars were used to test the fermenting property of the organism and also to differentiate it thereby, if possible, from the colon bacillus. The media used were 2 per cent sugar broths neutral to phenolphthalein. Before inoculation the tubes were allowed to stand at room temperature for 12 hours. Two colon strains were used as comparatives: one, the laboratory strain, and the other, a strain isolated from feces. Loopfuls from 24 hours' cultures of each of the three organisms were inoculated into the fermentation tubes, and these incubated at 37° C. Readings were taken at intervals of 24, 48, and 72 hours.

For convenience the bacillus isolated from the cheese will be termed *Bacillus* 3B.

TABLE V.  
FERMENTATION COMPARATIVES. PER CENT GAS.

Hours	B. COLON FECES			B. COLON LAB.			BACILLUS 3B.		
	24	48	72	24	48	72	24	48	72
Xylose	10	25	30	10	25	30	20	30	30
Arabinose	15	25	30	15	30	30	15	25	25
Dextrose	30	30	30	15	15	20	25	25	25
Mannose	15	15	20	20	20	25	15	15	20
Galactose	15	15	25	15	20	20	25	25	25
Mannite	50	70	70	15	30	30	40	60	60
Dulcitol	-	-	10	-	30	50	5	30	40
Lactose	30	40	40	30	40	40	30	40	40
Maltose	10	30	30	15	40	40	15	30	30
Saccharose	-	-	-	5	40	40	20	25	30
Raffinose	-	-	-	15	50	60	10	25	30
Inulin	-	-	5	-	5	5	-	-	-
Dextrin	10	10	15	5	10	10	5	10	10
Starch	-	-	-	-	5	5	-	-	-
Glycogen	-	-	-	-	-	-	-	-	-

It is evident that the fermentation method can not be used as a means of distinction between the *B. colon* and bacillus isolated from the cheese. The latter readily ferments the pentoses, hexoses, di- and trisaccharides. Of the polysaccharides it ferments only dextrin. It was thought, for a time, that its nonfermenting of inulin might be of help in distinguishing it from *B. colon*. Further comparative tests, however, showed that this was not feasible, since the laboratory and feces strains did not always ferment it, and that at times a bubble or two of gas would be formed in tubes inoculated with the bacillus from the cheese.

With the exception of Vaughan and Perkins other investigators did not attempt to study the fermenting properties of their organisms to any great extent. Holst<sup>17</sup> and Kühn<sup>19</sup> merely report that their organisms fermented lactose. Fonteyne<sup>18</sup> found both dextrose and lactose to be nonfermented. Vaughan and Perkins<sup>16</sup> showed that their bacillus fermented dextrose, lactose, saccharose, maltose, dextrin, starch, and glycogen.

## NITRATE REDUCTION AND INDOL FORMATION.

The bacillus reduces nitrates to nitrites, but to a smaller degree than the colon bacillus. Using the same strength of nitrate solution the reduction for the *Bacillus* 3B is two-fifths that for *B. colon*. It also forms indol.

## PATHOGENICITY.

When first isolated the bacillus proved to be pathogenic to mice only. Injections of the original cheese suspension into guinea pigs and rats had no effects. One c.c. of a pure culture of the organism grown in milk killed a mouse, when injected intraperitoneally, in 8½ hours. The organism was recovered from the heart blood, grown in milk, and again injected into a mouse. The mouse died within 10 hours. A rat injected with the same culture (1 c.c.) showed symptoms of being unwell, but did not die. After passage through three more mice the organism had increased in virulence to kill a rat. A guinea pig inoculated with the same culture that killed the rat was unaffected.

The organism was then passed through four rats, and after the last passage was isolated from the heart blood, plated on agar, and obtained in pure culture. It was then grown in milk for 24 hours at 37° C. and 1 c.c. injected, intraperitoneally, into a guinea pig. Death occurred in about 20 hours. The bacillus was then passed through two successive guinea pigs and again isolated in pure culture. Owing to the rush of other work the agar slant was left in the incubator (37° C.) for about 25 days; and at the end of that time it was found that the organism had lost its fermenting power and also its pathogenicity.

It was thought that possibly, owing to its selective media, the bacillus would gain in virulence by repeated transplanting into milk or lactose broth. Such was, indeed, the case. A tube of lactose broth was inoculated with the organism from the stock culture, incubated at 37° C. for 24 hours, and then transplanted into lactose broth. After five successive transplants the organism was found to be again pathogenic to guinea pigs.

That the lactose increased the virulence of the organism seemed evident. Dextrose had a similar effect. A tube each of bouillon, lactose bouillon, milk, and an agar slant were inoculated with the bacillus, incubated at 37° C. for 24 hours, and then 1 c.c. from each of the cultures injected, intraperitoneally, into guinea pigs, the agar slant being taken up in 10 c.c. of physiological salt solution. The pigs injected with the lactose bouillon and milk died in 8 hours; that with the bouillon culture, in 20 hours; while the pig injected with the suspension from the agar slant did not die until 28 days later. The explanation undoubtedly lies in the fact that an anaerobic condition, produced by the presence of sugars, increases the pathogenicity of the organism.

Occasionally some animals seemed to be refractory to seemingly fatal doses. Rats, especially, would not always show the same susceptibility. In order of susceptibility can be placed mice, guinea pigs, rabbits, cats, rats, and dogs.

The bacillus was passed through twenty guinea pigs and then isolated from the heart blood of the last pig. It was found that the virulence of the organism had increased so that .25 c.c. of a 24 hour lactose culture would kill a 200 gm. guinea pig within 20 hours. Intravenous injections did not seem to indicate any greater virulence than intraperitoneal. Subcutaneous injections were also fatal.

## POSTMORTEM APPEARANCE OF GUINEA PIGS.

The picture presented by a guinea pig killed with a fatal dose of the organism was usually that of a marked peritonitis. As a rule the lungs were slightly distended, the heart in diastole, and filled with blood. The liver, kidneys, and spleen were normal, although the latter at times was dark and slightly enlarged. The amount of peritoneal fluid present was variable, ranging from none to 5 or even 6 c.c. Usually it was present in an excess from the normal. This fluid was highly toxic, and killed cats which were not killed by lactose broth cultures of the organism. In most guinea pigs there was marked inflammation at the point of injection.

## DISTRIBUTION OF THE ORGANISM.

The bacillus is found in the heart blood, lungs, kidneys, liver, spleen, bile, and the peritoneal fluid. In greatest numbers, and in practically pure culture it is found in the peritoneal fluid. In the liver and heart blood it is present in small number. It is numerous in the spleen, kidneys, lungs and bile.

## EFFECTS ON CATS.

On September 29, 1916, a cat which had been starved for 24 hours was injected, I.P., with 4.5 c.c. of a 24 hours' lactose broth culture. The cat soon showed signs of unrest, and attempted to vomit. She remained in a comatose condition with high fever, the temperature reaching 41.6° C. 35 hours after injection. She recovered on the second day. The temperature of normal cats was then established previous to a series of tests as to their febrile reactions with the organism. Four cats were used, the temperature taken once daily for four days.

TABLE VI.  
NORMAL TEMPERATURE OF CATS.

DATE	TIME	CAT 1	CAT 2	CAT 3	CAT 4
Oct. 4	10.00 A.M.	38.05	38.15	38.65	38.10
5	11.30 A.M.	38.45	37.80	38.40	38.80
6	2.45 P.M.	39.10	38.40	38.80	39.50*
7	10.30 A.M.	38.30	38.45	39.00	37.90
Average Temperature		38.47	38.20	38.71	38.26
Average Temperature of the four cats.....		38.41			

\*Not included in the average, as the cat was highly excited.

Several cats were then subjected to injections of the organism and their temperatures taken.

October 9. Cat 4. Weight 3250 gm. This cat was injected, I.P., with 5 c.c. of a 24 hour lactose broth culture, at 9:45 A.M. Retching, vomiting, and purging took place within half an hour.

Temp. at 9.45 A.M. 38.20  
10.45 " 39.62  
11.45 " 40.12  
1.40 P.M. 40.58

Temp. at 2.30 P.M. 41.18  
4.30 " 40.50  
Oct. 10, 9.30 A.M. 39.10  
12.00 " 38.50

The cat recovered.

October 10. Kitten 2. Weight 580 gm., was given, I.P., 3 c.c. of a 48 hour culture (lactose broth) at 9.45 a. m. Retching, vomiting, and purging took place in 35 minutes.

Temp. at 9.45 A.M. 37.12  
10.45 " 38.76  
11.45 " 40.15  
1.15 P.M. 39.78  
2.15 " 39.30

Temp. at 3.15 P.M. 39.16  
4.15 " 39.20  
5.15 " 39.50  
8.00 " 38.86  
10.00 " 38.44

The kitten recovered.

October 11. Cat 2, weight 2500 gm., was given I.P., 8 c.c. of an 18 hour milk culture of the organism at 9.15 A.M. Retching, vomiting, and purging took place in 30 minutes.

Temp. at 9.15 A.M. 38.12  
 10.15 " 38.80  
 11.15 " 38.42  
 1.00 P.M. 38.72  
 3.00 " 38.74  
 8.00 " 40.00  
 10.00 " 39.40

Oct. 12, at 10.00 A.M. 39.88  
 11.15 " 40.10  
 1.30 P.M. 39.58  
 3.30 " 39.10  
 4.45 " 39.62  
 Oct. 13 at 9.45 A.M. 38.04

The cat refused food for two days. It is interesting to note that with the milk culture a longer sustained fever was obtained than with the lactose broth cultures.

Allusion has already been made to the marked toxic effect of the peritoneal fluid of animals killed by the bacillus. This fluid was of a much higher potency than either the milk or lactose broth cultures.

November 25. Cat 1, which had recovered from a dose of the pure culture given on September 29, was injected, I.P., with 5 c.c. of peritoneal fluid from Rabbit 116, killed by a lactose broth culture of the organism. Retching, vomiting, and purging took place in 20 minutes. The animal was found dead on the morning of the 26th. At the autopsy the heart was found to be in diastole, the blood being fluid. The lungs were slightly distended; the kidneys, hyperemic; the spleen, normal; and the liver, somewhat pale. No fluid was found in the peritoneum, although the viscera were moister than normally. The intestines were pale. The bacillus was recovered from the heart-blood and from the bile, and was shown to be present in smears made from the liver, lungs, spleen, and kidneys.

November 25. Cat 4. This cat was injected, I.P., with 2.5 c.c. of peritoneal fluid from a guinea pig killed by the bacillus. Retching, vomiting, and purging followed within half an hour, the vomiting and purging continuing, intermittently, for about 8 hours. The cat was found dead on the morning of the 27th. The autopsy showed the same picture found in Cat 1. The organism was recovered from the heart blood and bile, and was again shown to be present in the smears made from the liver, lungs, spleen, and kidneys.

December 1. Cat 2 was given, I.P., 3 c.c. of peritoneal fluid from a rabbit killed by the bacillus 3B. Retching, vomiting, and purging took place in 35 minutes.

Temp. at time of injection, 10.00 A.M. 37.00

11.00 " 37.60  
 12.00 " 37.48  
 1.15 P.M. 40.20  
 2.15 " 39.28  
 3.15 " 39.56  
 4.15 " 39.20  
 5.15 " 40.40

Dec. 2, 9.30 A.M. 38.48

The cat died at 5 P.M. on the 2nd. The postmortem findings were similar to those of the preceding cats. The bacillus was shown to be present in the same organs.

December 8. Kitten 1, weight 650 gm., was given, I.P., 2 c.c. of peritoneal fluid from a guinea pig. Retching, vomiting, and purging took place in half an hour.

Temp. at time of injection, 9.40 A.M. 37.32  
 10.40 " 39.00  
 11.40 " 39.00  
 1.30 P.M. 39.90  
 2.30 " 39.56  
 3.30 " 39.50  
 4.30 " 38.80  
 5.30 " 38.70  
 9.00 " 35.80

The kitten died late in the night. The postmortem findings were similar to those already described.



It is interesting to note that the temperature rose higher in the cats than in the kittens, although both were equally susceptible to the organism.

To summarize the tests with cats: The symptoms produced were retching, vomiting, and purging, generally within half an hour after injection. The stools were watery and copious. Extreme weakness so that the animal could not voluntarily stand up, and inability to eat food even after a previous starvation of 24 hours were characteristic. A marked fever was produced, the rise in temperature being marked the first few hours after injection. In all cases where the cats were injected with the peritoneal fluid death occurred; where given the lactose broth or milk cultures they recovered. Half-grown cats seemed to be the best adaptable for experimental purposes.

#### EFFECTS ON RABBITS.

Rabbits proved also susceptible to the organism. As with the cats the peritoneal fluid from animals killed by the bacillus was highly toxic to rabbits. The temperature did not reach the maximum obtained in the cats, although it showed an appreciable rise. In many of the rabbits there was a marked collapse, at times approaching paralysis. As a rule, large doses of the culture were necessary to cause death.

November 24. Rabbit 114 was injected, I.P., at 10.15 A.M., with 4 c.c. of peritoneal fluid from a guinea pig. Death occurred in 4 hours. The heart was in diastole; the blood, fluid. The blood vessels were distended. The organs appeared normal. There was very little fluid in the peritoneum.

November 24. Rabbit 116 was injected, I.P., at 9 P.M., with 4 c.c. of peritoneal fluid from a guinea pig. The rabbit was found dead early the next morning. The autopsy gave the same findings as those of rabbit 114, with the exception that there were about 5 c.c. of a dark fluid in the peritoneum. The bacillus was demonstrated in smears made from the lungs, kidneys, spleen, liver, heart blood, bile, and the peritoneal fluid.

December 1. Rabbit 133 was injected, I.P., with 1.5 c.c. of peritoneal fluid from a guinea pig. Temperature readings were taken at regular intervals.

Temp. at time of injection,	10.00 A.M.	38.35
	11.00 "	37.98
	12.00 "	38.38
	1.15 P.M.	38.72
	2.15 "	38.84
	3.15 "	39.82
	4.15 "	39.92
	5.15 "	39.40
Dec. 2,	9.30 A.M.	38.40

The rabbit died at 3.00 P.M. on December 2. The post mortem findings were similar to those already described.

Intravenous injections proved fatal when 1.5 c.c. of a 24 hour culture (lactose broth) were given.

December 8. Rabbit 160 was injected, I.V., with 1.5 c.c. of a 24 hour lactose broth culture at 10.30 A.M. The rabbit died early in the morning on the following day.

December 8. Rabbit 170 was injected, I.V., with 1.0 c.c. of a 24 hour culture (lactose broth) at 9.30 A.M.

Temp. at	9.30 A.M.	38.35
	10.30 "	39.30
	11.30 "	39.56
	2.00 P.M.	39.96
	3.00 "	39.48
	4.00 "	38.64
	5.00 "	38.90

The rabbit recovered.

## EFFECTS ON DOGS.

The effect of feeding dogs with the pure culture of the organism was tried. Finely chopped meat was saturated with a lactose broth culture of the bacillus and given to two dogs, who ate it with apparent relish. No ill-effects were visible.

December 13. A small dog, 3.5 Kg., was given, I.P., at 2 P.M., 5 c.c. of a 24 hour lactose broth culture of the bacillus. No retching nor vomiting, but purging was very marked. The temperature range for the period immediately after injection was as follows:

2.00	P.M.	38.3
3.00	"	39.5
4.30	"	40.1
5.30	"	40.1
7.00	"	39.2
8.00	"	38.8

The dog recovered slightly, but refused food or ate very sparingly. Death occurred early on the morning of December 19. Autopsy: The blood vessels of the mesentery and peritonum were enlarged. The heart was enlarged and the blood fluid. The vena cava was distended; the veins throughout the body were congested. The gall bladder was distended; the liver was large, with marked hyperstatic congestion. The lungs were normal. The kidneys were enlarged and hyperemic; when cut, they showed many bleeding points. The periphery was of dark color. The bacillus was recovered from the heart blood.

## EXPERIMENTS WITH MILK CULTURES.

With the view of determining whether tyrotoxinon was formed by the bacillus, experiments were conducted with milk cultures. The method of procedure was as follows:

Skimmed milk in 1000 c.c. portions was sterilized in the autoclave at 115° C. for 20 minutes. It was then inoculated with a loopful of the organism from a 24 hour agar slant, and incubated at 32° C. to 35° C. for 30 days. At the end of the incubation period the whey was separated from the curd by filtration through paper, and made sterile by filtration through a Berkefeld candle. The whey was then made alkaline with sodium hydrate and extracted with ether.

Vaughan and Perkins found that ether from certain commercial houses, on evaporation, left a residue which, when taken up in water and injected into guinea pigs, caused death. In all the work with ether alkaline extracts, therefore, controls were run on the purity of the ether. In no case was the ether found to contain a toxic residue.

The ether extract obtained from the whey was in the form of a gel, and when evaporated to dryness, left a caramel-colored residue, soluble in water. At no time were crystals obtained, and while the extract showed a slightly poisonous action, no tests made indicated the presence of tyrotoxinon.

The curdling of the milk, as a rule, took place within seven days; but the curd was, even at the end of a month, rather viscous and difficult to separate from the whey. The whey was always strongly acid to litmus. Intraperitoneal injections of the sterile whey into guinea pigs in 5 c.c. and 10 c.c. quantities had no effects. That the organism was not killed by its metabolic products were proved by inoculating bouillon tubes with a loopful of the whey; growth was always obtained, and sugars were fermented.

The alkaline ether extract, when taken up in water, gave a neutral reaction to litmus. In one or two of the portions the reaction was faintly acid. It had usually a rancid, almost putrid odor, and a slightly irritating taste. No positive

Biuret and Millon tests were given, although in one or two of the portions there seemed to be very faint positive reactions. In all of the extracts a strongly positive  $\alpha$  naphthol test was given.

The data in Table VII shows that the extract is slightly toxic. The alkaline ether extract was taken up in sterile water so that 10 c.c. represented the liter of the original milk.

TABLE VII.

THE EFFECTS OF ALKALINE ETHER EXTRACTS OF WHEY UPON GUINEA PIGS.

DATE	GUINEA PIG	INJECTION	AMOUNT	RESULT
Dec. 7	1	I.V.	2.0 c.c.	Death in 20 hours
7	2	I.V.	2.0 c.c.	Sick; recovered
7	3	I.V.	5 c.c.	Death in 48 hours
7	4	I.V.	7 c.c.	Death in 22 hours
8	5 to 10 inc.	I.V.	1 c.c.	No effects
8	11	I.P.	10 c.c.	Death in 7 days
12	12 to 14 inc.	I.V.	5 c.c.	Sick; all recovered

Attempts were made to obtain, if possible, the poisonous part in the extract by condensation through alternating freezing and thawing, a process used successfully in this laboratory by Ned R. Smith in his work on water and alcohol organ

TABLE VIII.

PROTECTION ACQUIRED BY GUINEA PIGS THROUGH ETHER EXTRACTS.

Oct. 27	G. P. 1 given	1 c.c. of extract, I.P.	No effects
Nov. 2	" " "	2 c.c. of a 24 hour culture of B. 3B;	death in 12 days
Oct. 27	" 2 "	1 c.c. of extract I.V.	No effects
Nov. 2	" " "	2 c.c. of a 24 hour culture of 3B;	death in 16 days
" 2	Control G. P.	1 c.c. of a 24 hour culture of 3B;	death in 12 hours
Oct. 27	G. P. 3 given	2 c.c. of extract, I.V.	Shock; recovered
" 30	" " "	1 c.c. of 24 hour culture of 3B;	death in 14 days
" 30	Control G. P.	1 c.c. of a 24 hour culture of 3B;	death in 12 hours
Dec. 8	G. P. 4 given	1 c.c. extract, I.V.	No effects
" 12	" " "	0.5 c.c. extract, I.P.	
" 21	" " "	1 c.c. of 24 hour culture of 3B.	No effects
" 8	" 5 "	1 c.c. extract, I.V.	No effects
" 12	" " "	1.25 c.c. extract, I.P.	
" 21	" " "	1 c.c. 24 hour culture 3B.	No effects
" 8	" 6 "	1 c.c. extract, I.V.	No effects
" 12	" " "	1.5 c.c. extract, I.P.	
" 21	" " "	1 c.c. 24 hour culture 3B.	Death in 4 days
" 21	Control G. P.	for G. P. 4, 5, 6 given 1 c.c. of a 24 hour culture 3B.	Death in 18 hours
" 12	G. P. 7 given	5 c.c. extract, I.V.	No effects
" 17	" " "	5 c.c. " I.P.	
" 21	" " "	5 c.c. " I.P.	
" 26	" " "	1 c.c. of 24 hour culture 3B.	No effects
" 12	" 8 "	5 c.c. extract, I.V.	No effects
" 17	" " "	5 c.c. " I.P.	
" 21	" " "	5 c.c. " I.P.	
" 26	" " "	1 c.c. of 24 hour culture 3B.	Death in 11 days
" 12	" 9 "	5 c.c. extract, I.P.	No effects
" 17	" " "	5 c.c. extract, I.P.	
" 21	" " "	" " "	
" 26	" " "	1 c.c. of 24 hour culture of 3B.	Death in 20 days
" 26	Control G. P.	for G. P. 7, 8, 9, given 1 c.c. of a 24 hour culture of 3B.	Death in 5 hours

extracts. The alkaline ether extract was taken up in sterile water, put into a freezing mixture, allowed to freeze, thawed at room temperature, frozen again, etc., until the solution showed two distinct layers, a colored and a colorless layer. The colored layer was pipetted off, and portions injected into guinea pigs. I.V. injections of the colored portions in 1 c.c. doses had no effects; 2 c.c. killed a guinea pig in 6 days; 2 c.c. of the colorless portion, injected I.P., killed a guinea pig in 4 days. On January 25, four guinea pigs were injected, I.P., with 1 c.c. each of the colored portion. Two died in three days, while the remaining two were unaffected.

A striking feature of the experiment with the alkaline ether extracts was the protection acquired by guinea pigs against fatal doses of the germ after they had been injected with the extract. This is shown in Table VIII.

Of the nine guinea pigs inoculated with the alkaline ether extract three were unaffected by fatal doses of the organism. The remaining six died after an interval ranging from four days to twenty days. The controls succumbed promptly from five to twenty hours. From these results it is evident that a fair degree of protection can be obtained against fatal doses of the organism, a protection which may approach immunity. Milk is not a good medium for the elaboration of the poison, but may become so under favorable conditions. The bacillus does not form tritoxicon.

#### FILTRATES OF LACTOSE BROTH CULTURES.

The marked pathogenicity of the organism when grown in lactose broth and the slightly toxic products obtained in milk cultures led to the study of the toxicity of sterile filtrates from lactose broth cultures. For the first test a broth containing 2% lactose and 5% glycerine was used. The broth, neutral to phenolphthalein, was inoculated with the bacillus, and incubated at 32° C. to 35° C. for 9 days. Several drops of tricresol were then added, and the culture filtered on the following day through a Berkefeld candle. The filtrate was tested for sterility.

On December 21 three guinea pigs were inoculated with increasing amounts: 0.5 c.c., 1 c.c. and 2 c.c. of the sterile filtrate. The pigs were restless for a short time but otherwise showed no ill effects. The filtrate was allowed to stand in a test glass, in a dark place, until January 17, 1917, on account of press of other work. Considerable condensation had taken place. When inoculated in 1 c.c. amounts into guinea pigs, intraperitoneally, it now proved fatal to two out of five of these animals, death occurring in 20 hours. On January 18 six more guinea pigs were inoculated with the following results:

G.P. 1	2 c.c.	I.V.	Death in 27 hours
G.P. 2	1 c.c.	I.V.	Death in 72 hours
G.P. 3	1 c.c.	I.V.	Death in 96 hours
G.P. 4	1 c.c.	I.P.	Death in 24 hours
G.P. 5	1 c.c.	I.P.	Death in 24 hours
G.P. 6	1 c.c.	I.P.	Recovered

The heart blood of each pig was found to be sterile. Marked inflammation of the intestines was characteristic in all cases. The organs were normal.

Tests were next made to determine whether the presence of glycerine was necessary or desirable. Three different broths were inoculated with the organism, incubated at 32° to 35° C. for 7 days, several drops of tricresol added, and the cul-

tures filtered on the following day through Berkefeld candles. The filtrates were then tested for sterility.

Broth A contained 2 per cent lactose.

Broth B contained 5 per cent glycerine.

Broth C contained 5 per cent glycerine, 2 per cent lactose.

TABLE IX.

COMPARATIVE TOXICITY OF LACTOSE, GLYCERINE, AND LACTOSE-GLYCERINE FILTRATES.

DATE	G.P.	AMOUNT INOC. L.P.	FILTRATE	RESULT
Jan. 31	1	1.0 c.c.	A	Death in 4 hours
31	2	1.5 c.c.	A	Death in 7½ hours
31	3	2.0 c.c.	A	Sick; recovered
31	4	1.0 c.c.	C	Sick; recovered
31	5	1.5 c.c.	C	Death in 4 hours
31	6	2.0 c.c.	C	Death in 6 hours
Feb. 1	7	1.0 c.c.	A	Death in 18 hours
1	8	1.5 c.c.	A	Death in 40 hours
1	9	1.0 c.c.	B	Sick; recovered
1	10	1.5 c.c.	B	Sick; recovered
1	11	1.0 c.c.	C	Sick; recovered
1	12	1.5 c.c.	C	Sick; recovered

The most toxic filtrate was obtained from lactose broth. It was then questioned whether the amount of sugar influenced the toxicity. To determine this point lactose broths of various strengths were made up, and the cultures grown and treated as already described.

Broth A contained .25 per cent lactose.

Broth B contained .5 per cent lactose.

Broth C contained 1 per cent lactose.

The guinea pigs used weighed from 200 to 225 grams.

TABLE X.

COMPARATIVE TOXICITY OF LACTOSE BROTHS.

DATE	G.P.	AMOUNT INOC., L.P.	BROTH	RESULT
Feb. 14	1	0.5 c.c.	A	Death in 5 hours
14	2	1.0 c.c.	A	Recovered
14	3	2.0 c.c.	A	Death in 48 hours
14	4	0.25 c.c.	B	Death in 7 hours
14	5	0.25 c.c.	B	Death in 12 hours
14	6	0.5 c.c.	B	Death in 3½ hours
14	7	1.0 c.c.	B	Death in 12 hours
14	8	2.0 c.c.	B	Recovered
14	9	0.5 c.c.	C	Recovered
14	10	1.0 c.c.	C	Recovered
14	11	2.0 c.c.	C	Recovered

Broth B, containing .5 per cent lactose, was most toxic, and this strength was used in the rest of the work with the filtrates.

The minimum lethal dose was determined, using varying quantities of the filtrate. One-tenth c.c. was found to be the smallest amount that would kill a 200-250 gm. guinea pig.

TABLE XI.

## DETERMINATION OF THE MINIMUM LETHAL DOSE.

DATE	G.P.	WEIGHT IN GRAMS	INOC. I.P.	RESULT
Mar. 12	1	176	0.1 c.c.	Death in 20 hours
12	2	200	0.1 c.c.	Recovered
12	3	203	0.1 c.c.	Death in 9 hours
12	4	208	0.1 c.c.	Death in 3 hours
12	5	216	0.1 c.c.	Death in 4 hours
12	6	224	0.1 c.c.	Recovered
12	7	197	0.01 c.c.	No effects
12	8	180	0.01 c.c.	No effects
12	9	203	0.01 c.c.	No effects
12	10	220	0.01 c.c.	No effects
12	11	238	0.01 c.c.	No effects
12	12	248	0.01 c.c.	No effects

Peculiarities early noticed regarding the toxin were: (1) Small doses invariably were more fatal than large. (2) Death occurred in a few hours. Thus a guinea pig receiving a dose of 0.1 c.c. would die in from 3 to 24 hours, whereas one receiving 1.0 c.c. or more would, in many cases, recover. The M.L.D. of this toxin can not very well be compared to the M.L.D. of either diphtheria or tetanus toxin, since the lethal period is so short.

## HEATED FILTRATES.

The toxicity of the filtrate demonstrated, it remained to determine whether the toxin was destroyed by heat,—in other words, whether the toxin was thermolabile or thermostabile. On March 21 a sterile filtrate was heated in a boiling water bath for ten minutes, cooled, and then injected into guinea pigs, intraperitoneally.

G.P. 1	Given 0.5 c.c.	Death in 8 hours.
G.P. 2	Given 0.5 c.c.	Death in 5 hours.
G.P. 3	Given 0.5 c.c.	Death in 4 hours.
G.P. 4	Given 1.0 c.c.	Death in 4 days.
G.P. 5	Given 1.0 c.c.	Recovered.
G.P. 6	Given 1.0 c.c.	Recovered.

The three pigs which had received 0.5 c.c. died within 8 hours, while only one out of three which had received 1 c.c. died, and that one in four days. Suspicion fell upon the use of the tricresol in the culture. Controls were, therefore, run using amounts even larger than in the cultures; but no ill-effects were ever observed. It was concluded that the toxicity was caused by the bacterial products, and was definitely proved by using filtrates to which tricresol had not been added.

On March 28 a sterile filtrate was divided into three parts: Part A was the normal, untreated filtrate; part B, the filtrate kept in a boiling water bath for 15 minutes; and part C, the filtrate kept in a boiling water bath for 60 minutes. When cooled to room temperature all three portions were injected, I.P., in varying amounts, into guinea pigs. The weights of the guinea pigs were, as in all this work, between 200 and 250 grams.

TABLE XII.  
TOXICITY OF NORMAL AND HEATED FILTRATES.

G.P.	INOC., I.P.	FILTRATE	RESULT
1	0.1 c.c.	A	Death in 4½ hours
2	0.25 c.c.	A	Death in 7½ hours
3	0.5 c.c.	A	Death in 5½ hours
4	0.25 c.c.	B	Death in 4½ hours
5	0.5 c.c.	B	Death in 18 hours
6	1.0 c.c.	B	Death in 8½ hours
7	0.25 c.c.	C	Death in 30 hours
8	0.5 c.c.	C	Death in 18 hours
9	1.0 c.c.	C	Death in 4½ hours

TABLE XIII.  
TOXICITY OF NORMAL AND HEATED FILTRATES.

G.P.	INOC., I.P.	FILTRATE	RESULT
1	0.1 c.c.	A	Recovered.
2	0.25 c.c.	A	Death in 5 hours
3	0.5 c.c.	A	Death in 5 hours
4	0.5 c.c.	B	Death in 18 hours
5	0.5 c.c.	B	Death in 5 hours
6	1.0 c.c.	B	Death in 4½ hours
7	0.5 c.c.	C	Death in 4 hours
8	0.5 c.c.	C	Death in 6 hours
9	1.0 c.c.	C	Death in 20 hours

As can be seen, the poison was not destroyed by heating, at boiling temperature, for one hour. This was corroborated by another series of tests, carried out as above, on April 10. The only change was made in the reaction of the media, where a -1 (1 per cent alkaline to phenolphthalein) was used instead of the neutral.

The postmortem findings were, as a rule, similar to those observed in the guinea pigs dying from the virulent culture. Marked inflammation of the intestines was characteristic in all animals dying from the filtrate, heated or unheated.

#### EFFECT OF FILTRATE UPON CATS AND RABBITS.

The sterile filtrate, when injected into cats, gave the same symptoms as the virulent culture, but was without fatal effects. The temperature increased as with the virulent culture.

February 15. Cat 7, weight 2250 gm., was given, I.P., 9 c.c. of a sterile filtrate from a .5 per cent lactose broth culture. Retching, vomiting, and purging took place within 45 minutes. The temperature readings were as follows:

Temp. at time of injection,	8.30 A.M.	37.0
	10.00 "	38.82
	12.00 "	39.64
	2.00 P.M.	39.82
	8.00 "	38.46

The cat recovered.

February 16. Cat 8, weight 2700 gm., was given, I.P., at 8.30 A.M., 9 c.c. of a 0.5 per cent lactose broth filtrate. Retching, vomiting, and purging occurred within 35 minutes.

Temp. at	8.30 A.M.	38.4	1.30 P.M.	40.42
	9.30 "	38.8	2.30 "	40.12
	10.30 "	39.0	3.30 "	40.28
	11.30 "	40.1	4.30 "	39.80
	12.00 "	40.1	5.30 "	39.3

The cat recovered.

A rise in temperature was the only effect produced in a rabbit injected with the filtrate.

February 15. Rabbit 180 was given, I.P., 5 c.c. of a 0.5 per cent lactose broth filtrate, at 8:30 A.M.

Temp. at	8.30 A.M.	38.5	11.45 A.M.	40.0
	9.00 "	40.02	1.45 P.M.	39.4
	9.45 "	39.70	8.00 "	38.08
	10.45 "	39.54		

The rabbit recovered.

#### FILTRATE FROM USCHINSKY'S SOLUTION.

It was considered that if the toxin had a definite chemical composition its study could be facilitated if it were formed in a synthetic medium. Uschinsky's medium was used. After inoculation with the organism and incubation for 7 days at 37° C., the solution was filtered through a Berkefeld candle and tested for sterility. Guinea pigs, injected with the filtrate, however, were not affected. This was not surprising in view of earlier work with cultures of the organism grown in this solution, when it was found that comparatively large doses were necessary to cause death in guinea pigs.

#### IDENTITY OF THE SOLUBLE POISON.

Attempts were made to isolate the soluble poison by the precipitation method, as with the toxins of diphtheria and tetanus; none, however, succeeded. The identity of the toxic agent remained, therefore, undetermined.

#### AGGLUTINATION.

The classification of the bacillus isolated from the poisonous cheese, from the study made thus far, would seem to place it in the *B. coli* group. The formation of a thermostable poison, however, indicates that the organism is not of the true colon type. To determine, if possible, whether a more accurate classification could be made, agglutination tests were carried out. Rabbits were immunized to *B. enteritidis*, *B. coli*, *B. paratyphoid A*, *B. paratyphoid B*, and *Bacillus 3B*, and their sera used in the tests.

TABLE XIV.

AGGLUTINATION OF *BACILLUS 3B* BY IMMUNE COLON, ENTERITIDIS, PARATYPHOID A, AND PARATYPHOID B SERA.

	1/10	1/20	1/50	1/100	1/200	1/300	1/500	1/1000	1/2000
Immune colon serum									
<i>B. coli</i>	++	++	++	++	++	++	++	++	++
<i>B. 3B</i>	++	++	++	++	++	+	-	-	-
Immune enter. serum									
<i>B. Enteritidis</i>	++	++	++	++	++	++	++	++	+
<i>B. 3B</i>	++	++	+	-	-	-			
Immune Para. A serum									
<i>B. paratyphoid A</i>	++	++	++	++	++	++	++	++	++
<i>B. 3B</i>	+	±	-	-	-				
Immune para. B serum									
<i>B. paratyphoid B</i>	++	++	++	++	++	++	++	+	-
<i>B. 3B</i>	++	++	+	-	-	-			



TABLE XV.

AGGLUTINATION OF B. COLON, B. ENTERITIDIS, B. PARATYPHOID A, B. PARATYPHOID B, AND B. 3B, BY IMMUNE 3B SERUM.

	1/10	1/20	1/50	1/100	1/200	1/300	1/500	1/1000	1/2000
B. colon	++	++	++	+	-	-			
B. enteritidis	++	+	-	-					
B. paratyphoid A	+	-	-	-					
B. paratyphoid B	++	-	-	-					
B. 3B.	++	++	++	++	++	++	++	++	++

A study of Table XIV shows that the bacillus isolated from the cheese, designated as B.3B, was agglutinated by immune colon serum in a dilution of 1/300; by immune enteritidis serum, in a dilution of 1/50; by immune paratyphoid A serum, in a dilution of 1/10; and by immune paratyphoid B. serum, in a dilution of 1/50. The bacillus, therefore, belongs to the colon group. This is further corroborated by the data of Table XV. Of the four organisms (exclusive of B.3B) tested, B. colon was agglutinated in the highest dilution by the immune 3B serum. That the dilution did not exceed 1/100 was somewhat surprising in view of the fact that immune colon serum agglutinated the 3B bacillus in a 1/300 dilution. The relationship of the organism, as shown by its agglutination, to the enteritidis and paratyphoid B. bacilli, may explain its formation of a soluble thermostabile poison.

It is to be regretted that no agglutination test could be carried out with the sera of the patients, none being available. Such tests have been carried out by several investigators. Hughes and Healey,<sup>26</sup> in their report of the Aldershot epidemic, stated that a colon bacillus isolated from the liver and kidney from a fatal case gave no reaction with the blood sera from other cases. No reaction was also given by the blood sera from several of the cases with Gärtner's bacillus. Berg<sup>28</sup> isolated the B. Paratyphoid B from the intestines and organs from a fatal case of cheese poisoning. This bacillus was agglutinated by the blood serum of the wife of the dead man, herself a victim of cheese poisoning. Fonteyne<sup>18</sup> found that samples of blood taken ten days after the accident did not agglutinate the bacillus he had isolated from the cheese. This bacillus was not agglutinated by sera immune to B. Aertrycke, B. de Bruges, and B. Typhoid. Serum immunized to the cheese bacillus agglutinated B. Aertrycke in 1/100 dilution; B. de Bruges, in 1/10 dilution; and B. Typhoid, in 1/250 dilution.

#### IMMUNITY.

In the immunization experiments, guinea pigs, on account of their susceptibility and adaptability to this kind of work, were used in preference to other laboratory animals. Immunization was obtained most satisfactorily by vaccine treatment. Agar slant of the organism, of 24 hours' growth, were washed with physiological salt solution, filtered through sterile cotton, heated at 58° C. for 1 hour, tested for sterility, and then standardized. Such a vaccine standardized on December 12 was found to contain 400,000,000 organisms per cubic centimeter. On December 13, six guinea pigs weighing from 200 to 220 grams each, were given, I.P., 5 c.c. of this vaccine. On December 17 a second dose of 1 c.c. was given; while the third dose, 1 c.c., was given on December 21. On December 26 all six pigs were given 1 c.c. of a 24 hour culture of Bacillus 3B. None

of the pigs died. A control guinea pig, injected with the same amount of the organism, died within 5 hours.

On February 4, 8 guinea pigs, weighing from 200 to 225 gm., were injected with .5 c.c. each of vaccine, made as above, and standardized so that 1 c.c.=250,000,000. A second injection of the vaccine, 1 c.c. was given on February 8. One of the pigs died on the following day. The third and final injection was given, in 1.5 c.c. amounts, on February 12. On February 19 all were given 1 c.c. of a 24 hour culture of *Bacillus* 3B. One guinea pig died on the 20th; the rest were unaffected. A control guinea pig, injected with .5 c.c. of the virulent culture, died in 16 hours. These tests show that a high degree of immunity is obtainable by vaccine treatment.

#### SERUM IMMUNITY.

The serum of guinea pigs immunized by several injections of vaccine, when injected into normal guinea pigs, immunized them to fatal doses of the virulent culture.

March 3. Two immune pigs were bled and their sera obtained aseptically. Four guinea pigs (210-225 gm.) were injected, I.P., with 0.1 c.c. of the immune serum. On March 8 one of the pigs was injected with 1 c.c. of a 24 hour lactose broth culture of B.3B. Death occurred 3 days later. A control pig died in 12 hours. On March 12, the second and third pigs were injected with 1 c.c. and 1.5 c.c., respectively, of a 24 hour lactose broth culture of B.3B. Guinea pig 3 died on the sixth day, while guinea pig 2 was unaffected. A control died in 12 hours.

Two guinea pigs injected, March 3, with .25 c.c. of the immune serum, were injected, on March 12, with 1 c.c. of a virulent culture of *Bacillus* 3B. Both were unaffected. A control died in 9 hours.

#### IMMUNITY TO SOLUBLE POISON.

While it was comparatively easy to obtain immunity to the virulent organism, it was very difficult to obtain immunity to the soluble poison it elaborated. Invariably the guinea pigs would die after the second or third inoculation, regardless of whether these doses were increased or decreased.

Thus, for example:

On March 12, three guinea pigs (200 gm.) were inoculated with .01 c.c. of a sterile lactose broth filtrate. On March 16 each received a dose of .1 c.c. of the filtrate. One died on the second, one on the third, and one on the fourth day after the inoculation. Again, on March 12, three guinea pigs were inoculated with .001 c.c. of the filtrate. On March 16 a second injection of .01 c.c. was given. One pig died on the 20th. The two remaining pigs were inoculated on the 24th, with .5 c.c. of the filtrate. These, as well as a control, died in 4 hours.

On March 21, six guinea pigs (200-250 gm.) were injected with .01 c.c. of the filtrate. On March 26, a second injection, .005 c.c. was given. One pig died on the following day, and one on the 29th. A third injection of .02 c.c. was given on April 7. One pig died on April 8. On April 14 the three remaining pigs and a control were injected with .25 c.c. of a freshly prepared filtrate. One of the pigs and the control died, the former in two days and the latter in seven hours. The other two guinea pigs were unaffected.

Guinea pigs immunized to fatal doses of the poison are immune to fatal doses of the virulent organism. Injections of heated sterile filtrate will immunize guinea pigs to fatal doses of the organism.

Two guinea pigs were injected, I.P., on March 3 with .5 c.c. of a sterile .25 per cent lactose broth filtrate. On March 12 they were given 2 c.c. each of .5 per cent lactose broth filtrate. The third dose, .2 c.c. of a .5 per cent filtrate, they were given on the 16th. On March 24 they were both injected with a freshly prepared .5 per cent filtrate, each receiving

.5 c.c. They were unaffected. A control died in 4 hours. On March 30 both were given 1 c.c. of a 24 hour culture of *Bacillus* 3B. They were unaffected, while a control, given the same dose, died in 15 hours.

On March 28 a .5 per cent lactose broth filtrate was heated, for one hour, in a boiling water bath. Varying quantities were injected into guinea pigs.

G.P. 1, weight 300 gm., received .25 c.c. No effects.

G.P. 2, weight 313 gm., received .50 c.c. No effects.

G.P. 3, weight 330 gm., received 1.0 c.c. Sick; recovered.

G.P. 4, weight 313 gm., received 1.5 c.c. Sick; recovered.

On April 8 all four received .5 c.c. of the heated filtrate. No effects were observed in any of the animals. On April 17, they were injected, in varying doses, with a 24 hour lactose culture of *Bacillus* 3B.

G.P. 1 received 2 c.c. of the culture.

G.P. 2 received 1.25 c.c. of the culture.

G.P. 3 received 1.0 c.c. of the culture.

G.P. 4 received 1.0 c.c. of the culture.

All were sick, but recovered on the following day. A 350 gm. control pig, given 1 c.c. of the culture, died in 10 hours.

From the data given it is evident that immunity can be obtained by injections of the sterile filtrate not only to the soluble poison itself, but also to the virulent culture of the organism. The soluble poison is undoubtedly not a true toxin, but evidence has been given that it does form immune antibodies.

#### CONCLUSIONS.

The data and results obtained in this work warrant the following conclusions.

1. Six cases of cheese poisoning were caused by a toxicogenic bacillus.
2. Morphological and biochemical reactions, and agglutination tests prove that the bacillus belongs to the colon group.
3. That it is not a true colon bacillus is shown by its formation of a soluble thermostabile poison.
4. Poisoning can not be induced in animals either by feeding the poisonous cheese or by feeding with cultures of the isolated organism.
5. Mice, guinea pigs, rabbits, cats, rats, and dogs are susceptible, in the order named, to the bacillus.
6. The bacillus does not form tyrotoxin.
7. Five-tenths per cent lactose broth, neutral to phenolphthalein, is the medium most suitable for the production of the poison.
8. The soluble thermostabile poison produced by the bacillus is a toxin-like body, of unknown chemical constitution.
9. Immunity to the organism can be acquired by vaccine and immune serum injections. It can also be obtained by injections of the sterile, cell-free filtrate from lactose broth cultures.
10. A lesser but yet marked protection can be acquired by injections of alkaline ether extracts of whey from milk cultures.

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# FURTHER EXPERIENCE IN THE TREATMENT OF TYPHOID FEVER BY THE INTRAVENOUS INJECTION OF SENSITIZED TYPHOID VACCINE SEDIMENT\*

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A LITTLE over a year ago Chickering and I<sup>1</sup> reported on the treatment of over fifty cases of typhoid fever by the intravenous injection of typhoid vaccine, which results, with some additional cases, were presented before this Association a year ago. Since that time opportunities have been available for the study of two additional groups of cases: the first at the Presbyterian Hospital in New York City, made possible through the great courtesy of Warfield T. Longcope; and the second a group of twenty-eight cases occurring in the Providence Hospital, Oakland, California, and rendered available for this study by the kindness of Edward von Adelung. The latter group of cases is of particular interest not only as being considerable in number, but as giving us our first opportunity of dealing with epidemic typhoid. These twenty-eight cases occurred among a group of workmen employed in constructing the San Pablo dam in Contra Costa county, and were due to a well traced contamination of drinking water which led to the occurrence of some fifty-two cases of typhoid fever, that have been reported on from the epidemiological standpoint by Geiger, Macmillan, and Gillespie.<sup>2</sup>

The method we have employed in treating typhoid has already been detailed, but may be repeated in essentials here. Treatment has consisted in the intravenous injection of the Gay-Claypole sensitized vaccine sediment, a preparation obtained from multiple strains of the typhoid bacillus of local and general origin, so chosen as to represent the characteristic antigenic groups of the typhoid bacilli that have been tentatively separated by Hooker, whose work has since been confirmed by Weiss. The vaccine is further prepared by treating these strains of typhoid bacilli with a rabbit immune serum of high agglutinating titer, subsequently washing them, and then killing and precipitating them by means of absolute alcohol. This killed vaccine is then dried to constant weight and ground in a mechanical mortar until the bodies of the bacteria are thoroughly disintegrated. The endotoxic substances are extracted by means of carbolated saline and the supernatant fluid rejected, the sediment of ground bacterial bodies being alone employed for injection. The use of this sensitized vaccine sediment has been reported on elsewhere in reference to its use in prophylactic immunization.<sup>3</sup> It has been found that this vaccine may be injected intravenously in adults in doses beginning with 0.02 of a milligram and gradually increasing in some instances to 0.06 of a milligram, without any harmful effects, although such injections give rise to a characteristic train of symptoms which seem intimately connected with the favorable results obtained. Our experiments herewith reported represent 1200 injections, and it may confidently be stated that no harmful results in the cases of

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typhoid fever involved, either immediate or delayed, are referable to the vaccine.

A synopsis of the cases of which this is a report is given in Table I, which we may proceed to explain in some detail.

TABLE I.

Total cases examined .....	194
Nontyphoid cases .....	62
Cases of typhoid on clinical basis alone.....	8
Cases of proved typhoid.....	124
Cases of proved typhoid not treated.....	26
Cases treated as suggested.....	98

One hundred ninety-four cases of suspected or possible typhoid were examined by full laboratory methods, and of these cases 62, by agglutination tests, blood cultures, and, in some instances, stool and urine examinations, could not be identified as typhoid fever. All of these sixty-two cases could eventually be likewise dismissed on clinical evidence as well, as certainly not typhoid. There remain, however, eight cases which may have been typhoid fever on the basis of the clinical examination, which, however, it was impossible to confirm by laboratory tests; these cases are not included in our series, although two of them were treated in the manner described with beneficial results. There remain, then, 124 cases of typhoid fever that may be accepted as certain on the basis of both clinical and thorough laboratory examination. Every case of proved typhoid fever, with the twenty-six exceptions to be mentioned presently, was treated in accordance with the method to be fully outlined at a later point.

We should here perhaps explain and possibly defend our decision to treat every available proved case. It has been a frequent custom among clinicians who wish to investigate any new method of therapy to treat a certain number of cases in a given series by the method in question, leaving another group of cases of variable size under the routine previously adopted for the object of furnishing what have been regarded as controls. This procedure has been patterned after the methods ordinarily in vogue in animal experimentation, where truly controlled experiments are essential and convincing. It should be pointed out, we believe, that no group of human cases of typhoid fever or of any other disease can so be utilized except on the basis of a relatively large number of cases, where purely statistical methods of computation may be employed. Chance infection in typhoid fever in no way represents the methods of experimental precision which are possible in animal experimentation, and no two cases of typhoid fever exactly resemble one another. In the first place, the infecting dose when derived from various sources of contagion must be extremely variable, and even in the case of an epidemic, as in the San Pablo epidemic, which forms a part of our series and where the contaminating dose may be assumed to be somewhat similar in all the cases, the widest variation is found in the individual reaction to the infection, a variation based on the individual resistance natural or acquired. We regard, then, the use of alleged control cases in a study of this sort as fallacious, inasmuch as they do not really control at all, and should prefer to judge our results, first, on the basis of what actually occurs in each and every case following the treatment employed, and, secondly, by comparison of this group of cases, although limited, with other recognized standards in respect to mortality incidence, dura-

tion of the disease, and the percentage of occurrence of complications. We believe that judgment arrived at on this basis requires more convincing evidence, which we believe is fortunately present in this particular case.

Twenty-six of our cases of proved typhoid were not treated, either because it was impossible to treat them, or because we believed that any results obtained in them might lead to an incorrectly favorable interpretation. Thus, in nine of our cases the temperature had already begun to fall, or the patient was indeed already afebrile. Three of them really represented a post-typhoidal condition, osteomyelitis, for example. Five refused treatment. In three, intravenous injections could not be given. Concerning three, no record is obtainable, and in three more death occurred before the laboratory diagnosis had been arrived at.

It should be emphasized that in no case was treatment withheld on account of the seriousness of the patient's condition, a fact of significance in consideration of the low mortality obtained. In two at least of our six fatal cases the prognosis seemed inevitably fatal when treatment was begun.

We may consider for a moment the results of laboratory diagnosis in the ninety-eight cases that were actually treated. The diagnosis was based first on the agglutination test (Widal) in all instances, on blood cultures in ninety-four of the ninety-eight cases, and in many instances on detection of the typhoid bacillus in the stool and urine. The agglutination tests were made by the macroscopic method with the employment of formalin-killed cultures of the typhoid bacillus. We have stated in our previous communication that complete sedimentation of the added bacteria overnight, even in dilutions as low as one to ten, are, if not absolutely diagnostic of typhoid fever (or typhoid vaccination), at least strongly presumptive evidence of it. We may mention here that of sixty-seven control, nontyphoidal cases tested in connection with this series, the Widal was negative by this method in a dilution of one to ten in sixty-four instances (95.5 per cent). The three exceptions were liver abscess, bronchitis, and trichiniasis, in the first and last of which previous typhoid could by no means be ruled out. On the basis of the lower dilution 94.8 per cent of our cases gave a positive Widal at some time during the disease, or if a higher and usual standard of dilution of one to forty alone is acceptable, 91.8 per cent of our cases were positive. Blood cultures (made by adding 10 c.c. of blood to 200 c.c. of 10 per cent bile broth) were positive in our series in 71 per cent, and in 13 per cent only were blood cultures negative when more than one examination had been made. In 68 per cent of the cases both Widal and blood cultures were positive. In two cases alone of the ninety-eight were both Widal and blood cultures negative, in which instances diagnosis was based on the isolation of the typhoid bacillus from the stools or urine.

The intravenous injection of our vaccine produces a series of distinct symptoms which vary markedly in intensity with individuals and with the dose employed. The usual amount on initial injection has been 1.50 of a milligram (corresponding to 150 million bacteria), and a corresponding dose in children, who, as in prophylactic immunization against typhoid, react less markedly than adults to corresponding amounts. I shall later refer to the findings of other investigators who have used other vaccines intravenously and who have described

alarming or even dangerous symptoms following their employment. It should be noted, however, that our vaccine, owing to its sensitization and the removal of endotoxins, certainly gives less perturbing, and, so far as we have observed, no really untoward effect. It seems necessary to produce a moderate reaction in order to effect the desired result, and the dosage in successive inoculations has been increased slightly in order to produce a similar train of symptoms on each inoculation, which a continuance of the same dose usually fails to do. The injection of the vaccine intravenously is followed in from fifteen minutes to an hour by a chill, which lasts from a few minutes to ten or fifteen minutes. This chill, or shaking, is not accompanied by a feeling of coldness, but rather by a sense of involuntary, spasmodic, muscular contraction. The chill is accompanied by a rise in temperature of one to three degrees, which reaches its height within three hours after injection and then falls. There may be a rise of temperature without a chill or the reverse. The rise in temperature is accompanied by a leucopenia, which may fall as low as 2000 to 3000 to the cubic centimeter. The chill is accompanied by an increase in the pulse rate and may be accompanied by slight cyanosis, slight respiratory distress, and frequently a sense of discomfort.

The temperature reaches normal or subnormal in about twelve hours. This fall in temperature is accompanied by sweating, which may be profuse and last for several hours, relaxation, and usually general amelioration of such symptoms as headache, delirium, and the like. The patient often feels perfectly well, and this condition, even when transitory, seems beneficial. Coincident with the fall in temperature there occurs a rise in the leucocytes, which may reach as high as 40,000 and which are characterized by a relative polymorphonuclear increase. I shall later refer, in reviewing the work of others with similar methods, to the dangers that have appeared with slightly different vaccine preparations, and to the contraindications to such injections that undoubtedly exist. It may be repeated, however, that in our experience of 1200 injections reactions of this sort have never appeared harmful, either immediately or ultimately, and contraindications seem, in the light of increasing experience, to grow progressively fewer.

No detailed method of procedure can be prescribed for treating any given case of typhoid fever by this method. The best results seem to be obtained by provoking a distinct, but not too severe, reaction of the type outlined. The dose necessary to produce such a result varies markedly with the individual and the particular balance already established between the typhoid bacillus and the reaction antibodies in the host. The temporary drop of temperature to normal may become permanent and remain there, in which case no further injections are required, except for the prevention of relapse. If the temperature again rises over a period of two or three days, the injection should be repeated in slightly increased amount, and so on until the desired result is produced or further injections are judged futile. A considerable number of injections may be given with perfect safety. As many as fifteen or sixteen have been given in certain instances, but if no striking result is obtained following three or four injections at two or three day intervals, very little effect from the treatment may be expected.



It has seemed wise to us previously to separate our cases into three rather definite groups in respect to the results produced by the vaccine injections, and we see no reason to change this grouping in the light of further experience. We believe that this grouping, based on results produced, is a conservative one and by no means represents all the benefit that attends this method of treating typhoid fever. We have briefly referred to the fact that the temperature

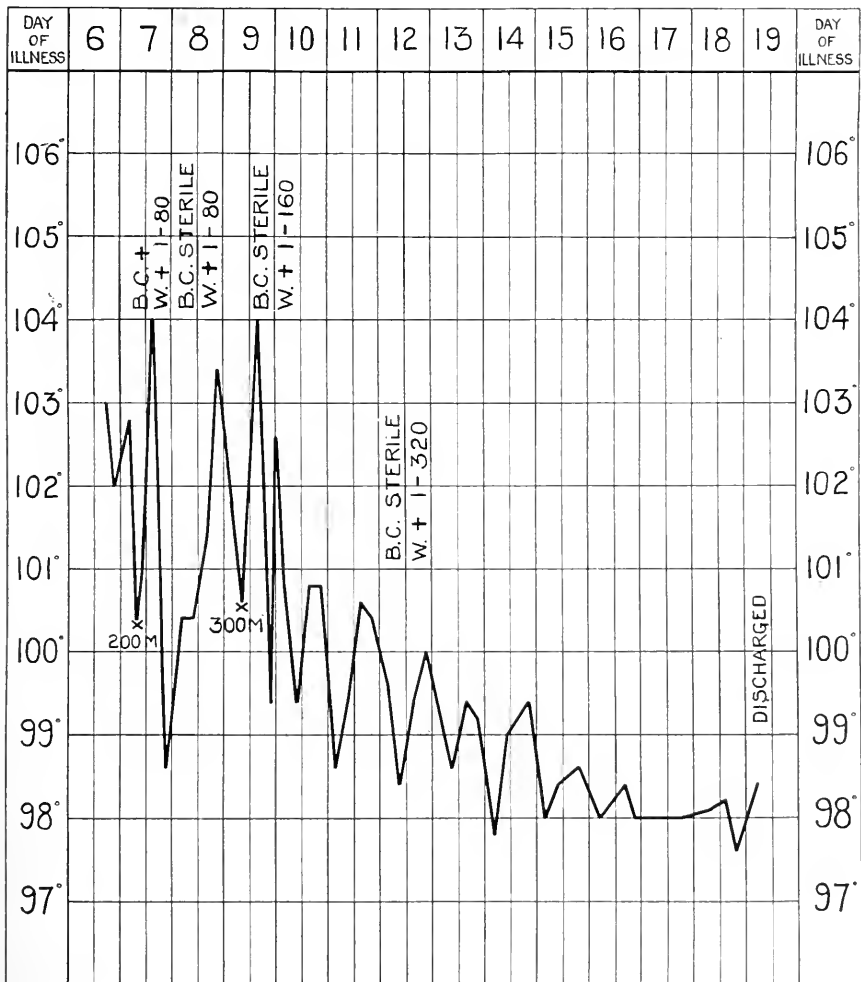


Fig. 1.—Case No. 35. Illustrating an abortive cure.\*

excursion with return to normal is accompanied by subjective feelings of well being, although at times of slight weakness, and would seem to be both immediately and ultimately of benefit to the patient. Such subjective amelioration is, of course, open to error in interpretation, but would seem, not only to us but

\*The data in these charts (Figs. 1, 2, 3) are abbreviated as follows: B. C., Blood Culture; W., Widal; A. T., Antityphoid serum; 75 M, Sensitized vaccine corresponding to 75 million typhoid bacilli. The temperatures indicated are the highest maxima forenoon and afternoon on each day by rectum.

to others who have watched our cases, to be frequent and of a convincing nature. The nurses, for example, in charge of the cases not only volunteer remarks as to the benefit produced by the vaccine injections, but emphasize the lessened care which patients treated in this manner entail. We prefer, however, to base our judgment as to the good results of the method rather on the more objective results that have been produced, the shortening of the duration of the fever, its frequent abrupt termination in an abortive form, the lessening of mortality and of complications in particular. We have classified the three groups of results obtained as abortively recovered, as benefited, or as unaffected,

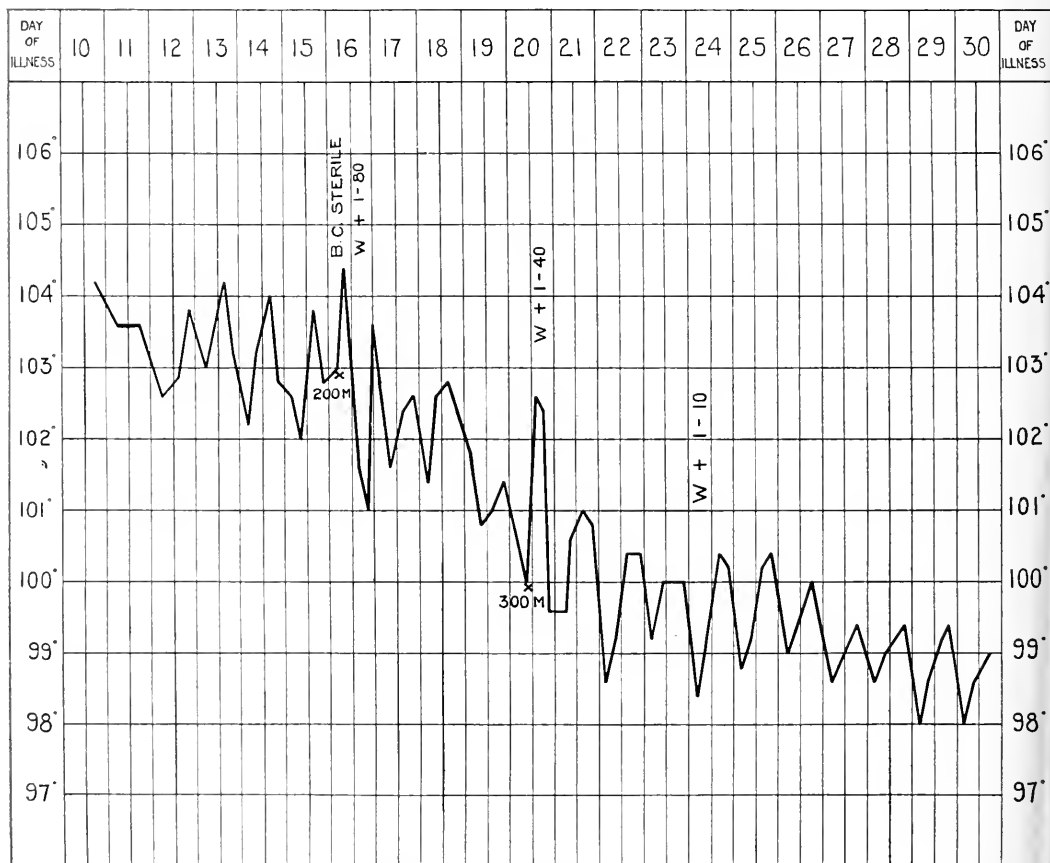


Fig. 2.—Case No. 43. Illustrating a benefited case.

and herewith present again charts from our previous article illustrating types of recovery of these three kinds. (Figs. 1, 2, and 3.)

A summary of our ninety-eight cases classified under these three headings proves of interest not only in respect to result achieved, but as bearing on the mechanism by which it is effected. In Table II are expressed certain characteristic findings in each of these groups of cases under consideration, which we may briefly refer to in drawing certain conclusions as to their significance.

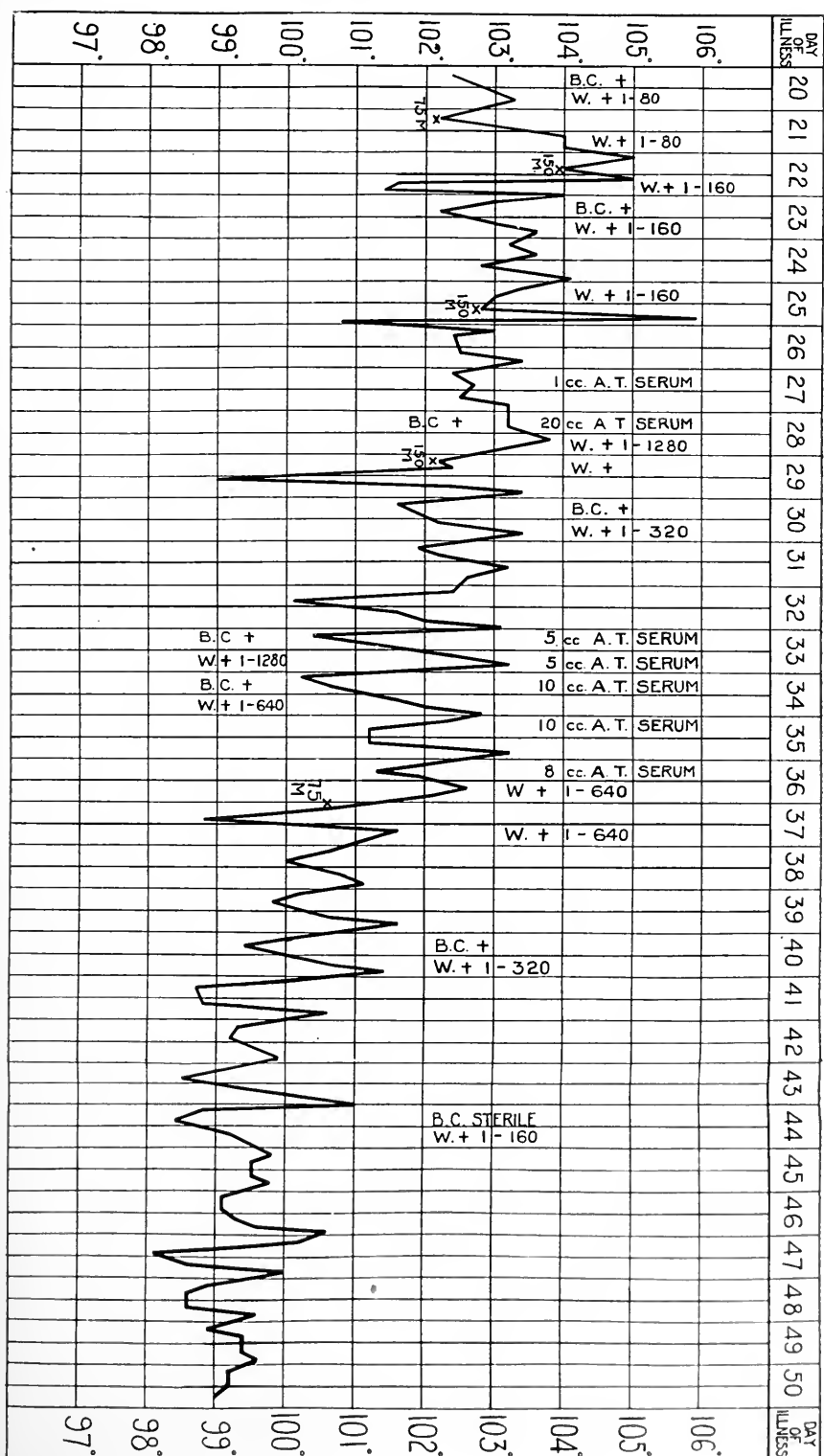


Fig. 3.—Case No. 47. Illustrating an unaffected case.

TABLE II.

SUMMARY OF RESULTS IN 98 CASES OF TYPHOID TREATED BY INTRAVENOUS INJECTION OF SENSITIZED VACCINE SEDIMENT.

	No.	Age	Widal Titer	B.C.	Treatment begun Day	No. of Treat- ments	Permanent Normal Temperature	Duration of Treatment
Aborted	33	26.2	296.0	36.6%	13.4	1.88	20.4	7.0
Benefited	32	24.2	156.5	70.9%	14.8	3.20	30.6	15.8
Unaffected	33	28.8	114.8	84.8%	13.7	4.85	46.8	33.1

In the first place, it will be seen that our cases may be divided into three almost exactly equal groups in respect to results produced. These groups, moreover, are of almost identical age, and it is also found that treatment was begun on the average at practically the same time in the three groups. They differ in practically all other respects. We find in the first place that antibody formation, as evidenced by the Widal or agglutinin titer in the cases before injections were begun, varies markedly in accordance with the eventual outcome of the case in question. In the abortively cured cases the Widal is found to be on the average over twice as high as in the unaffected cases, whereas the benefited cases lie intermediate between the two groups. A strong Widal is evidence, then, of a good prognosis, not only in respect to these treated cases, but probably also in untreated cases, to judge from the experience of others. Inversely correlative with the strength of the Widal is the occurrence of the percentage of positive blood cultures in the three categories under discussion; whereas the cases that respond less well to treatment are found to give positive blood cultures in nearly 85 per cent of cases, those that recover abruptly on treatment had positive blood cultures in only 36.6 per cent. The benefited cases again lie intermediate between the two. These relations between blood cultures and antibody formation we have already referred to as representing a balance established in the patient before intervention between the infecting agent and the resisting power of the host. When the resisting power is strongly developed, a favorable outcome may be more confidently expected.

This group of cases, which have been classified as abortively cured or abortively recovered, suggest in some respects the mild or abortive typhoid cases which are discussed in most extensive monographs on this disease. The typhus levissimus of Griesinger, the typhoidette of Brouardel, or abortive typhoid, forms a well recognized group or groups that must occur in every considerable collection of cases of typhoid fever. Estimates vary as to the exact proportion of such cases, as may normally be expected. It is probable, as Curschmann<sup>4</sup> suggests, that at least two groups should be formed, the mild typhoid fevers and the abortive typhoid fevers, the latter more frequently, though by no means invariably, characterized by a somewhat abrupt rise and fall of temperature and an abbreviated fastigium. McCrae in his 1500 cases found three per cent of mild forms of the disease, and, in addition, 0.1 of one per cent of his cases recovered by crisis. Letulle<sup>5</sup> found that these two types of the disease occurred particularly in certain epidemics and in children. He finds something over 17 per cent in the cases of the disease which he collected.

This figure may certainly be regarded as an extreme maximum of occurrence. According to Curschmann, the abortive and mild forms frequently have only three to six days of fever and almost never over ten or twelve days, with which statement Hare and Beardsley<sup>6</sup> would agree. At all events, the cases of mild and abortive typhoid together must constitute a relatively small percentage of all, in spite of the figures of Letulle. Hare and Beardsley note that Coleman found only twenty-four such cases in five years' experience at the Bellevue Hospital in New York. At all events, no such number as one-third of all the cases, as in our series, could be found in this way without some type of intervention. It may further be noted that our cases almost invariably have occurred beyond the limit of ten to twelve days set by Curschmann, and have practically invariably been characterized by a critical fall of temperature, and what is more important still, show a direct relation between this critical fall and the injection of vaccine. Our cases do not represent the mild epidemics of Letulle, nor has any considerable number of them been furnished by children. They have been gathered over three seasons and in widely different localities. As may be anticipated, the abortively recovered cases represent, by and large, the milder cases of the disease in this series as estimated before treatment was begun. Our characterization of a preponderance of these abortively cured cases as milder has no relation, however, to the small group of mild and abortive forms that have just been discussed. We have endeavored to register our impression as to the severity of each case before treatment was begun, and the results are expressed in Table III, where it is found that many more severe cases occur among those that remain unaffected by treatment, and many more mild cases occur among those that are aborted, whereas the benefited cases would seem to lie intermediate between the two.

TABLE III.  
GENERAL CONDITION OF CASES BEFORE TREATMENT.

	SEVERE	MODERATE	MILD
Unaffected	26	6	1
Benefited	10	17	5
Aborted	4	13	16

It is evident, then, that the milder cases react better to treatment than the more severe, but even the most severe ones will in some instances at least be benefited and even aborted. We believe the effects actually produced are best represented by the figures given under "Permanent normal temperature" and "Duration of Treatment" in Table II. It is found that a permanent normal temperature was obtained in the third of the cases that are called aborted on an average at about the twentieth day. When one considers that the treatment of these cases was not begun until the thirteenth day, the really significant figure of seven days as the average duration of treatment required to restore the patient to an essentially normal condition, so far as the temperature is concerned, is obtained. We find, moreover, that it took on the average a little under two injections to produce this result. The fall of temperature by crisis, which is exemplified in the Fig. 1 used to illustrate the abortive cure, is characteristic of

this category, although the abrupt fall may not occur in every instance on the first injection.

The benefited cases, again, are well typified in Fig. 2; each successive injection of vaccine produces a permanent fall in temperature on the average of one degree, and it may require several, on the average a little over three injections, to reduce the temperature to a permanent normal. The average duration, then, of these cases is found to be 30.6 days, and the duration of treatment the more significant figure, 15.8 days.

The cases that have been listed as unaffected, although showing the ordinary temperature excursions following each injection, are characterized by a prompt return of the temperature to its original or nearly its original height. The impression has remained in the minds of many who have seen these cases that they are really benefited, but since we have taken the reduction of temperature as our criterion of results, we prefer not to emphasize any good effect that may have occurred in these cases, beyond the diminution in complications and mortality, to which we shall presently refer. Even in this group of cases the average duration is by no means extraordinary, 46.8 days, although it does exceed somewhat, perhaps, a normal average duration of cases of typhoid fever. It is extremely difficult to estimate what such an average duration usually is. Curschmann regards the mild cases as lasting on an average of twenty-one to thirty-three days. In McCrae's<sup>7</sup> figures the average febrile period was thirty-one days. It should be noted that our figures refer to the total duration of the disease from its initial symptoms, so far as they can be estimated from the patient's history, and not from the known duration of the fever itself. The group of cases, then, that was best affected may reasonably be regarded as having run a distinctly short course, and the average of all cases, which is about twenty-nine days, is not markedly different from McCrae's thirty-one days.

The mortality in this series of ninety-eight treated cases is 6.6 per cent, which, when one considers that the cases were treated over a period of three seasons under varying conditions of care in private houses, as well as hospitals, and, moreover, treated in different parts of the country, is certainly a very satisfactory figure. The average mortality is usually figured at 10 per cent. The cases of death were predominantly due to the accidents of typhoid, three to hemorrhage, complicated with perforation in two instances, two to a combination of laryngitis and bronchopneumonia, and one only to typhoid toxemia.

The complications, again, were few in number, and only thirteen in all in the entire ninety-eight cases (13.2 per cent). This means, not that thirteen cases showed complications, for there were indeed only eight cases which showed complications, but the total number of complications was thirteen. These complications were as follows: pyelitis one case, lobar pneumonia one case, laryngitis two cases, bronchopneumonia two cases, toxemia one case, hemorrhage four cases, perforation two cases. The percentage of hemorrhage (4%+) and of perforation (2%) is certainly below what may be expected as the usual averages for these complications; 5 to 10 per cent for hemorrhage and 3 to 4 per cent for perforation. Although the earlier study of vaccine therapy in typhoid certainly led to the im-

pression, particularly when the vaccine was administered subcutaneously, that complications were diminished (Krumbhaar and Richardson,<sup>8</sup> Callison<sup>9</sup>), more recent work would seem to show that no distinct effect on the complications can be expected by the more recent intravenous type of treatment (Szecsy,<sup>10</sup> Wiltshire,<sup>11</sup> Reiter,<sup>12</sup> and Guinon and Malarte<sup>13</sup>).

The relapses are of particular interest in this series of cases because they show what we believe to be the effect of a method of treatment which we suggested in our previous article, and which has since been emphasized by others. It seemed from our previous work that relapses would be less likely to occur if the intravenous injections were followed, when the temperature had returned to normal, by a set of three subcutaneous injections on alternate days of the regular prophylactic dose (800 million bacteria or 0.1 milligram of our sensitized vaccine.)\* This method of treatment has likewise been recommended by Meyer,<sup>14</sup> Meyer and Altstaedt,<sup>15</sup> and Wiltshire.<sup>11</sup> There were ten relapses (10.2 per cent) in our series which may be compared with the usual average of 11.4 per cent given by McCrae. Eight of our relapses, however, occurred in cases in which the intravenous injections were not followed up by the subcutaneous treatment, so that when we come to divide the cases in relation to this treatment into two categories, we find that relapses occurred in only 5.39 per cent of the subcutaneously treated cases (37) and in 11.59 per cent of the cases in which intravenous injection alone was given (69). We believe, then, that this method should be followed in every case.

#### SUMMARY OF RECENT LITERATURE ON VACCINE THERAPY OF TYPHOID.

We may next consider the very considerable number of articles which have dealt with different methods of vaccine treatment of typhoid fever in the past few years. We do not intend at this point to repeat a historical account of the development of vaccine therapy in this disease, but wish here to give a summary of the results in very recent years, roughly from 1913 to 1917, both as indicating the extent to which these investigations have been pursued, but more particularly as indicating which of these methods is best. We have summarized in Table IV the results obtained by a considerable series of observers who have used different, or in some cases several methods of vaccine treatment.

TABLE IV.†

SUMMARY OF RESULTS OBTAINED BY RECENT OBSERVERS (1913-17) IN THE TREATMENT OF TYPHOID FEVER BY VACCINES ADMINISTERED IN VARIOUS WAYS.

	OBSERVERS	TOTAL CASES	ESTIMATES BASED ON	BENEFITED	MORTALITY
Untreated vaccine subcutaneously	30	1001	512	46%	14.5%
Sensitized vaccine subcutaneously	14	593	239	69%	8.0%
Untreated vaccine intravenously	22	501	233	62%	13.0%
Sensitized vaccine intravenously	12	487	316	85%	11.0%

\*These vaccine injections in the convalescent typhoid will in a few instances cause a rise in temperature of a degree on the same or the following day.

†The authors referred to in this summary are herewith given, a division being made in respect to the type of vaccine employed and its method of administration, as in the table. (1) Recent authors reporting

It will be noted, first, that the most frequent method of vaccine treatment in typhoid has been the subcutaneous injection of ordinary and usually heat-killed, untreated typhoid vaccines. The thirty authors on whose work we base our estimates, it should be repeated, are only very recent writers on the subject and by no means indicate the extent to which this method has been employed in previous years, a summary of which may be found in some of the articles quoted. This method of treatment, however, still remains somewhat the predominating one. Later modifications that have been introduced are, first, the use of sensitized vaccine subcutaneously, and then the injection intravenously either of plain vaccine or of sensitized vaccine. We have endeavored to estimate the results obtained by each of these methods of treatment. It is obvious that any such estimate is arrived at with difficulty. In the first place, many authors give wholly indefinite accounts of even the number of cases that they have treated, and even when the number is mentioned, the results obtained are frequently referred to as good, bad, or indifferent. The figures in the third column, on which the estimates of the percentage of benefit derived from each method of treatment is based, represent all the cases in the total number considered, in which a definite statement of result by the author was made.

One thing stands out preeminently in a consideration of this total recent literature, and that is that the great majority of the authors believe the vaccine treatment, irrespective of the method pursued, to be of benefit. The very few individuals who remain unconvinced of the value of the treatment or mention dangers in its employment have based their opinion on very few cases and a corresponding inexperience in the use of vaccine (Zupnik,<sup>16</sup> Peutz,<sup>17</sup> Sladek and Kotlowski,<sup>18</sup> Deutsch<sup>19</sup>). It seems further distinctly indicated from this table that the degree of benefit, and, to some extent, the reduction of mortality, would seem to increase with each successive innovation that has been suggested in recent years. The best results unquestionably seem to have been obtained by the intravenous injection of sensitized typhoid vaccine, the sensitization having been either by the use of convalescent serum, as advocated by Ichikawa, or by the use of a vaccine similar to the Besredka living typhoid vaccine. The superior results of sensitized vaccines and of intravenous injection over other methods is further evidenced when we consider those authors who have used more than one of the methods outlined. Thus, we find that Löwy, Lucksch and Wilhelm<sup>20</sup> obtained better results in their cases by giving plain vaccine intravenously than when it was given subcutaneously. Similar conclusions were drawn by Ortiz, Acuna and Belloc.<sup>21</sup> Paltauf,<sup>22</sup> Holler,<sup>23</sup> and Feistmantel<sup>24</sup> all conclude that sensitized vaccines produce better results than unsensitized vaccines.

on the use of untreated vaccine subcutaneously: 1911, Fletcher, 1912, Sadler, Callison; 1913, Fornet; 1914, Josne and Belloir, Weil, Sacquépée and Chevrel, Guinon and Malarte, Pensuti, Daretti; 1915, Goldscheider and Aust, Bourke, Evans and Rowland, Feistmantel, Mertz, Peiper, Krumbhaar and Richardson, Groer, Wiltshire and MacGillycuddy, Löwy, Lucksch and Wilhelm, Kahn, Reiter, Meyer, F., Brach and Fröhlich, Boselli; 1916, Lemanske, Peutz, Whittington, Zupnik, Müller and Leiner, Waitzfelder. (2) Authors who have employed sensitized vaccine subcutaneously: 1912, Ardin-Delteil, Negre and Raynaud; 1913, Ardin-Delteil, Negre and Raynaud, Roques, Boinet; 1915, Petrovitch, Ardin-Delteil, Negre and Raynaud, Garbat, Feistmantel, Szeeszy, Liebermann, Fellner, Löwy, Lucksch and Wilhelm, Boral, Deutsch; 1916, Galambos, Mayer. (3) Authors who have injected untreated vaccine intravenously: 1913, Thirloix and Bardon; 1914, Kraus and Mazza, Kraus; 1915, Biedl, Dithorn and Schultz, Csernel and Marton, Reihmayr, H., Mazza, McWilliams, H. I., Holler, Paulicek, Meyer, Rhein, Leutz, Ortiz, Acuna and Belloc; 1916, Fagnoli, A., Petzetakis, Zupnik, Müller and Leiner, Löwy, Lucksch and Wilhelm. (4) Authors who have injected sensitized vaccine intravenously: 1912, Ichikawa; 1914, Meyer and Alstaedt; 1915, Biedl, Eggerth, Holler, Sladek and Kotlowski, Koranyi, Landsberger, Löwy, Lucksch and Wilhelm; 1916, Rohoni, Galambos; 1917, Caronia.



It remains now to offer what explanation we may as to the mechanism by which the unquestionably beneficial effects of vaccine therapy in typhoid are brought about, and, particularly, why the intravenous treatment is better and the sensitized vaccine superior to the unsensitized. These relative differences seem to emerge inevitably from the attentive consideration of the literature that we have given, and have, at least in part, been preceded or followed by theoretical explanations of heuristic value.

#### SPECIFICITY OF VACCINE THERAPY.

We were careful to state in our previous article that the method of therapy which we were advocating could by no means be regarded as strictly specific in view of the results that had been obtained by other investigators. These results have since been markedly extended and may now be more readily classified than a year ago. In the first place, it has been found that beneficial effects of a very similar nature and characterized by similar reactions to those described have been produced in typhoid fever by the administration of vaccines prepared from bacteria other than the *Bacillus typhosus*. Kraus<sup>25</sup> was the first to note that he obtained beneficial results on the intravenous injection of colon as well as of typhoid vaccines. Ludke<sup>26</sup> and Lucksch<sup>27</sup> both obtained results with colon vaccine, and the latter author obtained a favorable effect by gonococcus vaccines and by sodium nucleinate. Zupnik<sup>16</sup> has utilized a vaccine made from the mouse typhoid bacillus as less toxic and as useful as true typhoid vaccine. Reibmayr,<sup>28</sup> who has employed colon and cholera vaccines in typhoid, as well as typhoid vaccine, concludes that the former, although beneficial, are not as good as that derived from the specific microorganism.

In a similar manner it has been found that other acute infectious diseases may be beneficially affected by the intravenous injection of their corresponding vaccines or of other vaccines. Thus, Kraus<sup>29</sup> notes the beneficial effect produced in cases of puerperal sepsis and pyocyaneus infections by the use of a colon vaccine. Rhein<sup>30</sup> notes the beneficial effect in paratyphoid fever of typhoid vaccine. Nolf<sup>31</sup> utilized peptone solutions advantageously in streptococcus infections. Miller and Lusk<sup>32</sup> have employed typhoid vaccine by intravenous injection advantageously in acute, subacute and chronic arthritis.

This leads to a consideration of the less complex proteins and, indeed, simple inorganic salts that have been employed with claim to equally favorable results both in typhoid and in other acute infections. Ludke<sup>26</sup> has described the use of deuterio-albumose in typhoid, Weichardt<sup>33</sup> the employment of albumin solutions, and Nolf, as already mentioned, has used one-half a gram of peptone dissolved in 200 c.c. of normal saline and administered intravenously. Baradulin<sup>34</sup> has utilized dextrose solutions in considerable amount in cases of surgical sepsis, and Weichardt and Engländer<sup>35</sup> have obtained similar results with normal physiological saline alone. The use of colloidal gold (*Colibiase*) by Letulle and Mage,<sup>36</sup> Barachon,<sup>37</sup> Gay,<sup>38</sup> Labbé and Moussaud,<sup>39</sup> and Delbet<sup>40</sup> would seem simply another instance of the nonspecificity of the reaction that we have described.

It should be reiterated that in each and all of these instances the beneficial effects produced, which are undoubted, are brought about by the *intravenous* injections of a nonspecific vaccine, a less complex protein, or even a complex or

simple organic or inorganic substance. These injections, moreover, have all been characterized by the train of symptoms we have described with typhoid vaccines, of chill, temperature excursion, and the results in the nature of critical recovery have been similar, though probably differing in relative percentage.

#### MECHANISM OF CURE IN INTRAVENOUS VACCINE THERAPY.

This proof of the nonspecificity of the most favorable results that can be produced by a typhoid vaccine in typhoid fever should not only not discourage us but lead us to inquire further into the mechanism of the reaction on which this benefit depends. In our previous work (Gay and Claypole, Gay and Chickering) we have been led to attribute significance to two factors, which we believe are not only concerned in, but are of importance in the reaction which is produced by intravenous vaccine injections. The first of these factors is the hyperleucocytosis which following our observations has also been found to occur by a number of authors (McWilliams,<sup>41</sup> Holler,<sup>23</sup> Löwy, Lucksch and Wilhelm,<sup>20</sup> Lucksch,<sup>27</sup> and Rohonyi<sup>42</sup>). It has also been shown that many of the substances which in a non-specific manner may lead to the same effects as, for example, nucleon (Melnikowa and Wersilowa<sup>43</sup>), and colloidal gold (Busquet,<sup>44</sup> Barachon<sup>37</sup>), also increase the leucocyte count, to which fact their results have been attributed.

As a second factor on which the degree of benefit obtained would seem to depend is the antibody content of the patient. This we have already brought out in previous work and repeated in this article, where it is found that the strength of the Widal titer seems to vary directly with the benefit that results from injection. This observation, moreover, would agree with the incidental observation of a number of observers that prognosis in general is more or less dependent on the strength of antibodies in the patient, and, particularly, that the prognosis of vaccine injection likewise bears some relation to the degree to which the individual has reacted against the infection. Of like significance is the extremely mild and favorable course of typhoid fever in the vaccinated. Koranyi<sup>45</sup> has found that the opsonic index rises after vaccine injection in typhoid, and Inez Smith in our laboratory has apparently been able to demonstrate a distinct relation in the degree to which the opsonic index increases after intravenous injection and the result produced. In other words, the opsonic index rose much more markedly in the cases that were abortively cured than in either the benefited or the unaffected cases. We have frequently found that the agglutinin titer rises after the vaccine injection.

Our previous hypothesis as to the mechanism by which cure was effected in the most striking instances was, briefly, that it was due to a cooperation between the leucocytes that were called out by the injection and the antibodies already present in the patient, which latter substances acting as tropines caused the digestion and destruction of the typhoid bacilli in the body by the increased white blood corpuscles. We gave several reasons for regarding this mechanism as probably apart from the now proved relation of the antibody strength and the leucocytic increase to the result obtained. In the first place, it can be shown by blood culture in some, though by no means all, instances that the bacteria disappear rapidly from the peripheral circulation following the injection. Similar results

have been obtained by other investigators. This working hypothesis may or may not be the correct or ultimate explanation of the results produced by this non-specific reaction in typhoid fever and in other acute infections. There are other explanations which are probably no more ultimate and which, indeed, are not of necessity in any direct conflict with the one we have presented. Jobling and Petersen<sup>46</sup> have attributed the results produced by the reaction as due to a disturbance of ferment, antiferment balance, the antiferment being absorbed and the ferment being allowed to act. This explanation is certainly a very attractive one. Teague and McWilliams<sup>47</sup> in recent experiments on rabbits also bring forth certain results which must be taken into consideration in explaining this type of therapy. They find that the injection of vaccine in rabbits produces a refractory condition which renders these animals temporarily more resistant to infection with the typhoid bacillus. They would attribute the results produced in the favorable cases of treatment to an overflowing of bactericidal substances, which are found to be present in the circulating blood, into the remoter lymph spaces which serve as metastatic foci in typhoid fever, and the consequent destruction of the bacteria that are present in them.

It makes very little difference whether we regard the leucocytes or bactericidal ferments in the serum as the cause of the ultimate destruction of the bacteria, with which recovery seems to be associated. It is probable, indeed, that the white blood cells are the sources of the ferments, and we see no reason why these various factors and experiments that have been brought out by Teague and McWilliams, Jobling and Petersen, and ourselves will not eventually stand in harmony in the ultimate explanation which may soon come.

In our previous article we expressed the hope that it might be possible to increase the percentage of most favorably reacting cases by supplying the hypothetical lack of antibodies, which seems to be characteristic of the unaffected cases. We mentioned two or three cases in which we apparently were able to produce abortive cures, although the antibody content was low, by injecting an immune serum followed by vaccine. We have since continued these experiments, but it must be confessed with no very encouraging result. In the first place, it seemed necessary to determine the best type of protective or curative antiserum that could be produced. We have utilized goats for the purpose and immunized individual animals on the one hand with sensitized, and on the other with unsensitized living typhoid polyvalent vaccines. It was found possible to compare the protective value of such sera with considerable accuracy by injecting them subcutaneously in mice with a subsequent intraperitoneal injection of typhoid bacilli grown on blood media. No striking differences in the protective value of the unsensitized as against the sensitized sera was evident. We then tried the effect of such sera in a few human cases of typhoid and found that the serum alone usually produced the same type of nonspecific reaction brought about by the vaccine. A great deal of work has recently been done in France on the use of antityphoid serum of this general type, particularly the serum advocated by Rodet. In a series of articles from 1910 to the present time Rodet and his collaborators<sup>48</sup> have described the preparation of an antityphoid serum in horses and the results produced by it. Similarly favorable results have also been ob-

tained by Remond and Manvielle<sup>49</sup> and Étienne.<sup>50</sup> A survey of their results, however, does not lead one to believe that the results with the serum even when given intravenously are as good as those produced by means of vaccine, and, indeed, the results produced would seem due rather to the reaction which the injection causes, and which differs only in degree from the reaction produced by vaccine, than to any antibodies that are present in the serum. Abortive cures are reported by these observers; Étienne, for example, in 12 cases out of 200, and Rodet in a somewhat larger percentage, but these abortive cures would seem to be less frequent than when vaccine is employed, and the shortening of the disease, although distinct, is by no means as pronounced as by the use of vaccine.

In view of the nonspecificity of the characteristic reaction on which the vaccine treatment of typhoid depends, it may be questioned why we continue to advocate typhoid vaccine in preference to any of the other substances that have been employed. To repeat our opinion of a year ago, we would say that typhoid vaccine alone can build up the specific immunity to the typhoid bacillus, on which, at least in part, the cure may depend, and with which a lessening of complications would seem certainly to be associated. Typhoid vaccine, moreover, when employed in the form of the Gay-Claypole sensitized sediment is a harmless substance, convenient to employ and active in very small amounts. Again, the degree of leucocytosis on which the result obtained would seem in part to depend is, we still believe, increased in a relatively specific manner when a sensitized vaccine is used. We are aware that McWilliams<sup>51</sup> has been unable to repeat some of our experiments on specific hyperleucocytosis. We are not prepared to state to what her failures were due.

Zinsser and Tsen<sup>52</sup> have found at least that immune animals react more markedly to bacterial injections than do normal ones. They do not, however, agree with us that this reaction is absolutely specific in that heterologous antigens will apparently produce the same effect. It is unfortunate that neither of these investigators has repeated our experiments, in which we found that normal animals reacted more markedly to sensitized vaccines than to untreated vaccines.

#### SUMMARY AND CONCLUSIONS.

This article records our experience in the treatment of ninety-eight authenticated cases of typhoid fever over a period of two and one-half years by the intravenous injection of a polyvalent sensitized typhoid vaccine sediment (Gay-Claypole vaccine). The cases were observed for the most part in hospitals, but to some extent in private houses. The series includes cases studied in New York City, as well as in California. We regard the treatment as indicated in any case of typhoid fever that is still febrile, on the basis of our results which seem excellent. The mortality in these cases was low (6.6 per cent) and the complications few. Relapses were distinctly reduced in those cases in which the intravenous injections were followed by a series of three subcutaneous inoculations after the temperature had reached normal.

Our estimate of the beneficial results which have followed this treatment, apart from the probable reduction of mortality and complications, is based on the following facts:

1. In one-third of the cases the temperature fell critically following the first or second injection, and a permanent normal was established on the average within a week after beginning treatment.

2. In a second series of cases each injection was followed by a permanent decrease of a degree or more in the temperature.

3. Subjective benefit, apart from temperature changes, seemed to follow the majority of inoculations.

The treatment is perfectly harmless with the particular vaccine and under the conditions employed.

A detailed consideration of similar methods of therapy that have been studied with increasing interest in the last two years would seem to show that intravenous injections are far superior to subcutaneous injections of vaccine, and that sensitized vaccines are better than plain typhoid vaccines. It appears that the reaction is not strictly specific in that a similar type of reaction, chill, temperature excursion, etc., which is characteristic of the intravenous vaccine injections, may be produced by similar administration of other vaccines, of other proteins, and, indeed, of salt solution. There is some evidence, however, that relatively better results are produced by the use of the typhoid vaccine which in addition aids in establishing an active immunity against the specific micro-organism concerned. The exact mechanism by which the benefit or cure is effected is as yet uncertain, but it is at least marked by a temporary increase in the number of leucocytes and is probably dependent on the strength of antibodies already present in the individual patient. Our efforts to supply these antibodies by means of an antityphoid serum have not met with great success, and the beneficial effects that others have obtained with antityphoid serum would seem to be due to a mechanism similar to that following vaccine injections, but less effective than the latter.

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## THE RELATION OF PHYSICAL CHEMISTRY TO THE IRRIGATION OF WOUNDS\*

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SO many factors enter into the healing of a wound that it is very difficult to make an analysis of the response to treatment in any moderate number of cases. It seems permissible, therefore, to discuss certain physiological principles which should be kept in mind in any investigation of the methods of treatment. If a wound can be sterilized, it may be closed, and hence is outside the present discussion. Since the object of the irrigation of wounds is the removal of bacterial toxins and necrotic tissue as long as the wound remains infected, the irrigation must be continued for a long time. The prolonged diffusion between the irrigating fluid and underlying tissues necessitates unusually detailed precautions for the protection of these tissues. Although the ultimate sterilization of the wound is the object of the treatment, no drug has been found that will kill all classes of bacteria embedded in tissue without injuring the tissue, and hence time must be allowed for the development of antibodies. For this reason the antiseptic properties of the irrigating fluid must be subordinated to its physiological properties.

During the past ten years, I have investigated the effects of various media on many classes of cells. Not all of this work has been published, but the main points, as well as references to the literature and special methods of work may be found in my book.<sup>1</sup> My early work was done on bacteria, moulds, protozoa, egg cells and embryos. For instruction in the preparation of tissue cultures of warm-blooded animals, I am indebted to Montrose Burrows, with whom I had the good fortune to be associated for several years.

Conditions which impair the functions or kill cells may be roughly divided into radiations, changes in temperature, and mechanical, osmotic, and chemical changes, the last including the effects of the electric current. Some cells are

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more resistant than others, and their relative resistance to different classes of adverse conditions varies greatly. Thus some cells are unusually resistant to osmotic changes and quite sensitive to chemical changes. As a rule a cell that is sensitive to one chemical change is proportionately sensitive to another, the few exceptions being in relation to specific drugs. The ratio of the concentration of one substance to another may have to be considered in the case of those having antagonistic actions.

Electrolytes are dissociated into ions, and it is usually the electro-positive ions that are most toxic, the order being about as follows, commencing with the least toxic: Na, Cs, Li, Rb, K, Mg, Ca, Sr, Ba, Al, Mn, Sn, Fe, Tl, Co, Ni, Zn, Pb, Cd, As, Cu, Bi, Sb, Hg, Ag, Pl, Pt, Au. H and OH ions kill most rapidly owing to their great speed of diffusion, but when the time element is not considered, they must exceed certain concentrations in order to be toxic and are difficult to place in the series. This question will be considered below in connection with special methods for the estimation of the concentration of H and OH ions. To facilitate the slow administration of an ion it may be combined in an organic compound which slowly liberates it, as As in salvarsan and cacodylic acid, Ag in argyrol, and Hg in  $K_2Hg(CN)_4$ . Na, K and OH ions antagonize the action of Ca, Mg, and H ions. The antagonism between H and OH is complete because they combine to form  $H_2O$ . The antagonism between Na and Ca is almost complete but it requires but a little Ca to antagonize a large amount of Na. The other antagonisms, and many not mentioned, are less complete. The alkali-metals are similar to one another in action, but only Na and K have been thoroughly studied. As a rule K is more toxic to human cells, but Na is more toxic to many cells of lower animals and plants. The antagonism between  $HgCl_2$  and NaCl is due to the formation of the poorly dissociated  $Na_2HgCl_4$ , and holds only where the exposure is of short duration.

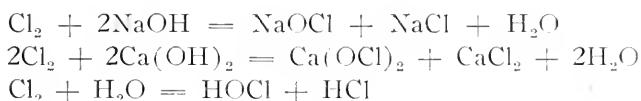
The toxicity of the indifferent anesthetics is proportional to their effect in lowering the surface tension of water, and hence the toxicity of their solutions is proportional to the number of drops that fall from the end of a pipette after one filling. The dropping surface of the pipette should be about 5 mm. in diameter, and the delivery tube constricted so that at least a minute is required for all of the fluid to drop out. Not only the substances used for anesthesia, but a larger number of substances including ethers, alcohols, ketones, esters, soaps, nitriles, halogen hydrocarbons, benzol derivatives, alkaloids and many other substances, fall in this class. Some of these substances which are not sufficiently soluble for antiseptics may be beneficial to wounds, as for example, iodoform, which increases the activity of phagocytes.

Chemically active substances, such as phenol and cresol, and especially those with powerful oxidizing or reducing action, may also lower the surface tension of water; but are far more toxic than would be expected from the surface tension of their solutions. Formaldehyde does not owe its toxicity entirely to its reducing action, but forms additive compounds with proteins, which may decompose again with the liberation of formaldehyde. Oxidizing substances are, however, reduced by cells, and an ideal local antiseptic would be one whose reduced product is indifferent. Hydrogen peroxide falls in this class,



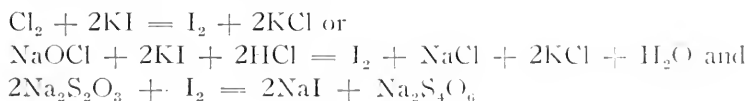
but is not a powerful oxidizing agent and is decomposed by catalase so rapidly as to render the large percentage of it ineffective. It acts chiefly as a mechanical cleanser. If infusoria are placed in a solution of  $\text{H}_2\text{O}_2$ , the latter penetrates their protoplasm and is decomposed on the inside with the liberation of bubbles of oxygen which burst and destroy the cells. More useful oxidizing agents are iodine and chlorine, especially the latter since the  $\text{HCl}$  formed on its reduction may be neutralized by  $\text{NaHCO}_3$  that has been added and thus rendered indifferent. According to Dakin and his collaborators, chlorine forms chloramines when it acts on protoplasm, and these chloramines have an antiseptic action. It is true, however, that chlorine oxidizes many organic compounds, with the liberation of  $\text{HCl}$ . Chlorine gas escapes rapidly from its solution in water, but this may be retarded by the addition of a base, transforming it into hypochlorite. Its oxidizing power is impaired, however, if the reaction is very alkaline, but may be restored by bubbling  $\text{CO}_2$  through the solution.

It makes little difference whether chlorine gas ( $\text{Cl}_2$ ) or calcium hypochlorite ( $\text{CaO}_2\text{Cl}_2$ ) or sodium hypochlorite ( $\text{NaOCl}$ ) or hypochlorous acid ( $\text{HOCl}$ ) are used in so far as their antiseptic action is concerned (except that the solution should not be strongly alkaline), as they are readily and spontaneously converted into one another according to the formulæ:



Since solutions of all these substances give off chlorine to the air and do not retain their original concentration, some rapid method of determining their concentration is useful. The most accurate method is titration with arsenious acid, since it is not affected by chlorate, and many refinements in iodimetric standardization may be used, but the following simple method suffices for the present purpose.

The process depends on the liberation of iodine by the chlorine and the titration of the iodine with sodium thiosulfate ( $\text{Na}_2\text{S}_2\text{O}_3$ ) commonly called "hypo" by photographers, the reaction being:



If specially pure sodium thiosulfate crystals, showing no efflorescence can not be obtained, a saturated solution in distilled water at body temperature is made and cooled until crystals form. These crystals are dried in air, and have the composition  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ . Of these crystals 24.832 grams are weighed and dissolved in distilled water that has been boiled to remove the  $\text{CO}_2$ , so as to make a liter of tenth normal solution. This solution must be protected from the  $\text{CO}_2$  of the air by means of soda-lime tubes, and is therefore more conveniently handled in an automatic burette. In making the titration, a few crystals of  $\text{KI}$  and about a cubic centimeter of  $\text{HCl}$  are placed in a flask and 10 c.c. of the chlorine

solution run in from a pipette, then titrated with the thiosulfate solution until the iodine color disappears. Dakin and Carrell state that the proper strength for wounds is .45 per cent to .5 per cent, but this is more conveniently stated as 0.127 to 0.141 normal, as it requires 12.7 to 14.1 c.c. of the thiosulfate to titrate 10 c.c. of the irrigating fluid that is of proper strength.

Apart from the chlorine or other antiseptic in the irrigating fluid, it should be physiologically normal. Suppose the chlorine were dissolved in distilled water, for example. The chlorine would be reduced or bound on meeting the first layers of tissue or exudate, but the water would penetrate deeper and injure the tissue without affecting the bacteria that had escaped the chlorine. If the chlorine were dissolved in an isotonic solution of NaCl, this salt in pure solution would be injurious and the HCl formed on reduction of the chlorine still more toxic, although both would be somewhat lessened in action by the admixture of exudate. In a hypertonic solution the exudate would be increased, but the hypertonicity is injurious to tissues.

Dakin's fluid is usually prepared from a solution of calcium hypochlorite by precipitating the lime with a mixture of carbonate and bicarbonate of sodium. Although  $\text{CaCO}_3$  is only 0.0013 per cent soluble in distilled water, Dakin's fluid usually contains more than this, and there is probably always enough to antagonize the toxicity of the Na. It is necessary, however, to add more than the physiological concentration of Na in order to precipitate the greater part of the Ca, and the solution is hypertonic. The freezing point of human blood is  $-0.56^\circ\text{C}$ ., but the samples of Dakin's fluid that I have tested froze at  $-0.7$  to  $-0.9^\circ$ , and were therefore very hypertonic. Even after precipitating a large part of the lime by bubbling  $\text{CO}_2$  through the solution, it was necessary to add so much  $\text{NaHCO}_3$  to precipitate the remainder of the lime that the solution was hypertonic. The precipitation depends on the alkalinity and the concentration of  $\text{CO}_2$  and other substances. Oxalic acid can not be used to precipitate the lime as the chlorine immediately oxidizes it to  $\text{CO}_2$  and the antiseptic action of the fluid is simultaneously lost. I have made isotonic irrigating fluids by running chlorine gas into physiological salt solutions in which the NaCl had been replaced by a chemically equivalent amount of  $\text{NaHCO}_3$ . The physiological properties of the salts will now be considered.

The physiological salt solutions in common use in laboratories are by no means perfect, as illustrated by the following comparison of their percentage composition with that of the diffusible salts in blood serum:

	NaCl	KCl	$\text{CaCl}_2$	$\text{MgCl}_2$	$\text{MgSO}_4$	$\text{NaHCO}_3$	$\text{NaH}_2\text{PO}_4$
Ringer-Locke	.9	.042	.024	—	—	.01-.03	—
Tyrode	.8	.02	.02	.01	—	.1	.005
Serum	.65	.04	.02	—	.012	.33	.013

In the data for serum, only the diffusible salts are included, and owing to ionization and recombination, a larger number of salts are present than indicated in the table. The expression of the data in this form is for comparison with physiological salt solutions. Tyrode has been the only previous investigator to introduce Mg into physiological salt solution and apparently no one but the writer has used  $\text{SO}_4$ . The formula for physiological salt solution successfully used by the writer is that given in the last line in the above table, and more

nearly imitates serum. Only one difficulty might arise. The blood is under a partial pressure of  $\text{CO}_2$  of 40 millimeters of mercury, or 6 per cent of an atmosphere, and when this pressure is relieved it becomes more alkaline, thus initiating coagulation and other changes. A solution of the blood ash would become alkaline in the same manner, and hence toxic to living cells with which it came in contact. A solution of diffusible serum salts should be tightly stoppered to prevent the loss of  $\text{CO}_2$ , or the  $\text{CO}_2$  thus lost should be replaced by bubbling the gas through the solution. The proper reaction may be reached by bubbling the alveolar air of a normal man through the solution. If pure  $\text{CO}_2$  is bubbled through the solution, some means must be used to ascertain when the proper reaction is reached. The most convenient way is to determine the hydrogen ion concentration, as given in the following paragraphs.

It is more convenient to express alkalinity (or acidity) in terms of the minus logarithm of the hydrogen ion concentration, abbreviated to PH. The PH of a neutral solution is about 7, whereas that of blood is 7.5, and that of sea water about 8. The extreme limits of the variation of the PH that is compatible with the life of most cells is from 5 to 9, and the majority of cells do not bear variations beyond 6 to 8. The writer made some experiments with species of mould sealed in glass tubes by fusing the glass so that no fresh spores could enter. The mould thrived in a saturated solution of boric acid containing 3 per cent pure NaCl; but if enough NaOH was added to bring the PH up to 8, no more growth took place (alkaline limit). Clark and Lubs<sup>2</sup> give the acid limits, of moulds and bacteria at 2.3—6, and alkaline limit 9.4.

The PH of any solution, free from  $\text{NH}_3$ ,  $\text{H}_2\text{S}$ ,  $\text{Cl}_2$  and certain antiseptics, may be determined by means of the hydrogen electrode, and the PH of many solutions may be determined approximately by means of indicators and solutions standardized by means of the hydrogen electrode. For solutions containing chlorine (Dakin's fluid) none of the ordinary methods may be used. The chlorine poisons the hydrogen electrode and destroys the indicators with extreme rapidity. The method used by the writer consists in selecting a series of indicators in aqueous solution and noting the color of the indicator solution the moment it is dropped into a large quantity of Dakin's fluid. The indicator solution must be brought to the yellow color before being used. If the resulting colors are recorded and compared with the following table, the PH may be determined approximately.

	3	4	5	6	7	8	9	10
Tetrabromphenolsulfonphthalcin	yellow	bluish	blue	blue	blue	blue	blue	blue
Methyl red	red	red	orange	yellow	yellow	yellow	yellow	yellow
Dibrom-o-cresolsulfonphthalcin	yellow	yellow	yellow	purplish	purple	purple	purple	purple
Dibromthymolsulfonphthalcin	yellow	yellow	yellow	yellow	green	blue	blue	blue
Neutral red	red	red	red	red	orange	orange	orange	orange
Phenolsulfonphthalcin	yellow	yellow	yellow	yellow	red	orange	red	red
o-Cresolsulfonphthalcin	yellow	yellow	yellow	yellow	yellow	orange	red	red
a-Naphtholsulfonphthalcin	yellow	yellow	yellow	yellow	yellow	green	blue	blue
a-Naphtholphthalcin	white	white	white	white	white	greenish	green	green
Thymolsulfonphthalcin	yellow	yellow	yellow	yellow	yellow	green	green	blue
Phenolphthalcin	white	white	white	white	white	white	pink	red
Thymolphthalcin	white	white	white	white	white	white	white	blue

Most of these indicators are more soluble in alcohol, but alcoholic solutions must not be used, owing to formation of  $\text{HCl}$ . Phenolsulfonphthalcin and a-naphtholsulfonphthalcin which will act only in aqueous solution and then very poorly, should be avoided if possible. These indicators are quickly attacked by

the chlorine and changed into yellow compounds which may lead to erroneous interpretation of the results unless careful observation is assured. Obviously not all of the indicators are necessary, and a choice may be made among those of the same range. If a physiological fluid is made of neutral salts, it can be brought to a PH of about 7.5 by the addition of 0.0035 per cent  $\text{NaHCO}_3$  and blowing air through until equilibrium is attained, after which it will not change on exposure to air, but has little buffer action against acid, and alkalies. If  $\text{CO}_2$ —free fluids are used, the PH may be adjusted by the addition of borate or phosphate buffer mixtures, but these will absorb  $\text{CO}_2$  slowly from the air and become less alkaline.

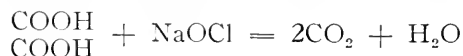
In case of solutions into which chlorine gas is to be run, practically all of the salt content should be bicarbonate, as the base is necessary for the formation of the hypochlorite and the  $\text{CO}_2$  for the reduction of alkalinity. The solution which I have used chiefly contains 1.25%  $\text{NaHCO}_3$ , 0.04%  $\text{KCl}$ , 0.02%  $\text{CaCl}_2$ , 0.025%  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , and 0.01%  $\text{NaH}_2\text{PO}_4$ .\* A brown glass bottle is filled with this solution and inverted in a basin of the solution, and chlorine gas is passed into the bottle through a glass tube until it displaces more than half of the solution. The tube is removed and the stopper inserted and the bottle shaken so that the solution will absorb the gas, the stopper being lifted occasionally so as to admit air in the space from which the gas has been absorbed. The solution is then titrated and if too strong, diluted with some of the gas-free solution.

If a cylinder of chlorine gas is not at hand, the gas may be generated as follows: A flask is about one quarter filled with powdered manganese dioxide and  $\text{HCl}$  added until well moistened. A perforated rubber stopper is inserted in the flask and connected with a bent glass tube to lead the gas to the bottom of the inverted bottle. When the gas ceases flowing it may be started again by immersing the flask in water and heating it to boiling. Still more gas may be obtained by the addition of more  $\text{HCl}$ . If a separatory funnel is at hand it may be used to introduce more  $\text{HCl}$  through a second perforation in the rubber stopper without lifting the stopper, but it is a matter of convenience and not necessary. As the chlorine bubbles out of the concentrated hydrochloric acid in the flask, some  $\text{HCl}$  gas is carried off with it, but this quantity is negligible if the first charge of the acid is entirely soaked up in the powdered  $\text{MnO}_2$  and no heat is applied at the start.

The PH of the irrigating fluid may be determined as indicated, and lowered by bubbling  $\text{CO}_2$  through, or raised by bubbling air through it. The PH should not be allowed to exceed the limits 5 to 7.5 unless it is desired to irritate the tissue. The PH depends on the ratio between  $\text{CO}_2$ , which tends to acidify the fluid, and  $\text{NaOCl}$ , which has an alkaline reaction due to hydrolysis according to the equation.



When an organic compound such as oxalic acid, for instance, is added to the Dakin's fluid, this alkaline hydrolysis is prevented, owing to the reaction:



\*This solution may be charged with chlorine by means of a sealed tube of chlorine now on the market.

According to Dakin,<sup>3</sup> when hypochlorite comes in contact with proteins the following reaction takes place:



and hence the solution would become more alkaline. In a mixture of proteins and other organic substances, reactions of both types may occur and tend to balance one another, neither increasing nor decreasing the alkalinity to a marked degree.

It should be noted that in both types of action of NaOCl on organic substances (in which oxygen or chlorine combines with the organic substance) the organic substance is oxidized. Oxidation, in the broad sense, is not combination with oxygen but an increase in the positive valences or decrease in the negative valences. For example, in the oxidation of HCl by manganese dioxide to form chlorine gas as described above, the electro-negative chlorine ions lose their negative charges (free valences) and become electro-neutral chlorine atoms as expressed by the equation:



in which the negative charges of the anions are expressed by (') and the positive charges of the cations by (·) and show that the negative charges of 2Cl' are transferred to O, thus enabling it to combine with 2H·.

According to Dakin, it is not the NaCl which sterilized the wound but chloramines formed by its action on organic substances, and he proposes to substitute a chloramine ( $\text{CH}_3\cdot\text{C}_6\text{H}_4\cdot\text{SO}_2\text{Na}\cdot\text{NCl}$ ) for the NaOCl. He states that chloramine does not give off chlorine in solution (and hence it has not the oxidizing power of NaOCl). This is in harmony with Dakin's statement that chloramine is not as effective as NaOCl in removing necrotic tissue. Dakin, Cohen and Kenyon<sup>4</sup> observed that guinea pigs would tolerate a subcutaneous injection of 1 gram of chloramine per kilo. From this it may be concluded that the oxidation products formed by the action of NaOCl on necrotic tissue are not unusually toxic if absorbed, and hence the advantage of NaOCl over phenol is obvious.

In order to prevent the absorption of bacterial toxins from the wound, Wright advocates the use of very hypertonic (5%) NaCl containing oxalate. It should be remembered, however, that this solution is toxic to adjacent tissues, and therefore it seems advisable to try other methods. If the flow of Dakin's fluid, for instance, is rapid enough, it should carry away these toxins to a large extent. Isotonic Dakin's fluid would be absorbed by the blood especially if there was a lack of sufficient water in the body. It has been shown by Starling and especially by F. H. Scott<sup>5</sup> that it is the osmotic pressure of proteins that enables the blood to absorb isotonic solutions, since the blood vessels are less permeable to proteins than to other substances. Only when the blood pressure in the capillaries equals the excess of osmotic pressure does absorption fail to occur. It has been shown by Hertzler, Murphy and others that absorption from the peritoneum can be prevented by the administration of large quantities of water to the patient, and this method might be used to prevent the absorption of an isotonic irrigation fluid from a wound. A continuous rectal irrigation with water might be used simultaneously with the irrigation of the wound with isotonic Dakin's fluid.

NaOCl as such has little oxidizing power, but its dissociation products are powerful oxidizing agents. Increase in the alkalinity of the solution decreases the dissociation of NaOCl and hence decreases its oxidizing power. The dissociation products are used up when the fluid comes in contact with tissue, thus leading to dissociation of more NaOCl and decrease in the concentration of the solution, finally to zero. It is obvious that rapid irrigation with a dilute solution will have the same oxidizing effect as slow irrigation with a more concentrated solution, and have the further advantage of more thorough washing out of the wound. This is true of the wound cavity, but when thick masses of necrotic tissue are acted on, the diffusion into them of NaOCl is regulated by its actual concentration on the outside, or in other words, penetration depends on concentration. Since, however, it is not desired that the NaOCl penetrate the living tissue, lower concentrations are preferable.

The writer has prepared low concentrations of NaOCl by a simplified method, as follows: Ringer's fluid is placed in a glass jar, and 2 electric light carbons inserted. A direct electric circuit is connected with the carbons and the current passed for about 15 minutes, when the NaOCl is titrated. The current need not be more than a few volts, and if the lighting circuit of 110 or 220 volts is used, it must be passed through a resistance so as to prevent overheating of the solution. Not all of the NaCl can be transformed into NaOCl.  $\text{CO}_2$  is passed through the solution until the required PH is reached. Heitz-Boyer has shown that such a solution can be conveniently made on board a hospital ship by the electrolysis of sea water. The writer has for many years (in case of emergency) used sea water diluted with 2 or 2.5 volumes of fresh or distilled water in place of Ringer's fluid. This solution contains an excess of magnesium but not sufficient to be toxic to tissues. The PH is about 8 and hence some  $\text{CO}_2$  should be added. Undiluted sea water is very irritating to wounds and delays healing, especially if some evaporation occurs. If the sea water is washed out of a wound, it heals more quickly even without the application of antiseptics. This does not favor the hypothesis that the toxic action of sea water on a wound is due to pathogenic bacteria in the sea water; it seems more probably due to its salts.

Dakin's fluid, made with the physiological precautions discussed in this paper, has been tried on large wounds in dogs and men. Not sufficient data have been collected to show that these precautions are always worth the trouble, but on the other hand, no disadvantages of such a solution have been noted. The theoretical considerations that led to the experiments recorded in this paper are given in the following discussion:

#### DISCUSSION.

Cell media or solutions coming in contact with living cells are of two physiological classes: nutritive and protective, as shown by the work of Sydney Ringer, J. Loeb, Osterhout, and many others. A great deal of speculation has arisen over the protective action of cell media and especially over the antagonistic or antitoxic action of ions on one another as evidenced by their combined effect on cells. The writer was the first to show by chemical analysis that the toxic action of ions and other substances on animal cells is associated with changes in permeability, and to quantitatively measure the diffusion of determined substances

from the cells on increase in permeability. Thus a quantitative and qualitative method of studying permeability and the toxic action of substances in the cell medium and the antitoxic action of other substances was built up. It had been shown long ago that increased diffusion of substances out of cells occurs on their death, and permeability had been supposed to be a means of regulating physiological activity. The writer has shown that the permeability of muscle increases on contraction and the permeability of eggs increases on fertilization, and if increase in permeability is not brought about by means of the sperm or other agent, no segmentation of the egg occurs. Fertilization of the frog's egg is associated with the outward diffusion of Na, K, Mg, Ca, Cl,  $\text{SO}_4$ , and  $\text{CO}_3$  (in addition to the  $\text{CO}_2$  or other products of metabolism). A greater than normal increase in permeability is associated with pathological changes or death. The permeability of certain fish eggs may be increased by certain alkaloids such as strychnine, by caffeine and by pure solutions of salts. If these eggs are placed in a toxic solution of quinine, for instance, the rate at which Na, K, Mg, Ca, Cl, and  $\text{SO}_4$  diffuse out of them is associated with their pathological condition. A certain increase in permeability is associated with the development of the eggs into one-eyed embryos or embryos with various defects in the nervous or circulatory systems. These abnormal embryos may hatch, but maximal increase in permeability is associated with death.

Physiological permeability is reversible, pathological permeability is not instantaneously reversible, and permeability associated with death is irreversible. Stimulation of cells consists in reversible increase in permeability. In general, methods used to kill cells, if carefully applied, may be used to stimulate them; i. e., to cause muscle to contract, or to cause eggs to develop without the necessity of the male element. This increase in permeability is associated with the very unstable surface layer of protoplasm. This layer may be compared with a copper ferrocyanide precipitation membrane. The latter will not allow sugar or  $\text{MgSO}_4$  to pass through it, whereas water passes freely; but if NaCl is added to the water, sugar will pass through the membrane. In other words, NaCl increases the permeability of the copper ferrocyanide membrane. Protoplasm is made up largely of colloids, and it seems probable that in its normal state the colloid particles on its surface are so close together that diffusion is impeded. Some cells do not allow water to diffuse in or out, while most cells are freely permeable to water and oxygen. The permeability to other substances varies with the cells and their physiological states. Anything that causes a rearrangement of the colloid particles of the cell surface alters its permeability. The electric current has this effect and is the most rapid means of stimulating or killing cells. Cells may be sensitized so that they are more easily stimulated or killed. This sensitization may consist in a subminimal increase in permeability so that a smaller increase than normally necessary will result in stimulation, or in some other change increasing the instability of the colloids. Anesthetics, when applied in certain definite concentrations, dissolve in certain of the colloid particles, thus increasing their size, or surround them with a protecting layer, thus increasing the difficulty of stimulation; but when the concentration of the anesthetic reaches the lethal dose for that cell, some of the colloid particles are dissolved out of the

surface layer by the anesthetic or changed in some other way so that the permeability is increased and death results. A certain degree of impermeability is necessary in order to preserve the integrity of the cell, as otherwise its soluble constituents would be lost, but certain special degrees of semipermeability are associated with the metabolism and functions of the cell, and facilitate the escape of waste products and the absorption of necessary substances.

Since the surface protoplasm of cells varies, their sensitivity to toxic substances varies. Pascucci showed that those red blood corpuscles containing the larger amount of lecithin are more easily rendered permeable (laked) by soaps and other substances which attack lecithin, whereas the reverse is true of those erythrocytes containing the larger amount of cholesterin. No doubt the protein constituent of the surface of the corpuscle affects the specificity of laking agents for blood of different species of animals, but even less seems to be known of the constitution of proteins than of lecithin and cholesterin. This form of specificity has not been sufficiently worked out to be applicable to many groups of pathogenic organisms. More success has been achieved in finding specific poisons for protozoa than for bacteria, and in general, bacteria seem to be more resistant than are tissue cells.

Permeability studies have given the clue to the antagonistic action of salts (ions) on one another. Any ionic species, when in sufficient concentration and unaccompanied by antagonistic ions, increases the permeability to such an extent that death occurs. Tissues of mammals seem to bear higher concentrations of Na ions than of any others, and tissues of marine animals seem not to be killed by great changes in concentration of Mg ions; but either of these, when maintained in absolutely pure solution, is deadly. Na<sup>+</sup> causes increase in permeability, and Mg<sup>++</sup> causes decrease in permeability; and hence they are antagonistic to one another when applied in the proper ratio, but a pure solution of Mg<sup>++</sup> of sufficient concentration causes increase in permeability. Within physiological limits, OH<sup>-</sup> causes increase in permeability and H<sup>+</sup> causes decrease in permeability, but a high concentration of H<sup>+</sup> causes increase in permeability. Likewise it is possible under certain conditions to show that Ca<sup>++</sup> causes decrease in permeability but the quantitative side of this question needs further investigation.

The ideal irrigation fluid for wounds seems to be one which will maintain the normal permeability of the tissue cells and at the same time wash out and sterilize the cavity of the wound and sterilize the surface of its walls, the antiseptic being one that will be used up and thus prevented from penetrating the living tissue. Since many proteins are soluble in salt solutions, necrotic tissue should break up if the formation of bacterial zoogloea are prevented. The greater the alkalinity (the farther from the isoelectric point of the proteins) the greater the solvent action, but the more irritating to the issue.

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# ANTIDOTES IN MERCURIC CHLORIDE POISONING\*

## SECOND COMMUNICATION

### THE VALUE OF PHOSPHITE AND HYPOPHOSPHITE COMBINATIONS

BY BERNARD FANTUS, M.D., AND EMRY G. HYATT, CHICAGO, ILL.

IN a previous study,<sup>1</sup> it was found that a mixture of sodium phosphite and sodium acetate (Carter's Antidote) and one of sodium hypophosphite and hydrogen peroxide gave the best results among all the antidotes experimented with. Inasmuch as the results with the antidotes named were approximately equal, as may be seen from Table I, reproduced here from the previous publication, it seemed of importance to determine which one of the two was the best for practical purposes.

TABLE I.

#### SUMMARY OF AVERAGE LETHAL PERIODS.

Mercuric Chloride given alone.	██████████
Mercuric Chloride followed by Stannous Chloride.....	████████████████████
Mercuric Chloride followed by Sodium Bicarbonate.....	██
Mercuric Chloride followed by Egg Albumen.....	██
Mercuric Chloride followed by Sodium Acetate.....	██
Mercuric Chloride followed by Sod. Hypophosphite with Acetate .....	██
Mercuric Chloride followed by Sod. Phosphite with Acetate.	██
Mercuric Chloride followed by Sod. Hypophosphite with Hydrogen Peroxide.....	██

#### CONTROL TESTS.

Owing to the importance of rigid control, one or more animals in each series were given the poison without antidote. The results are shown in Table II. We have now administered the dose of 0.04 gm. per kg. to 22 (oats and carrots fed) rabbits with a fatal result in all cases, and an average fatal period of seven and three-fourths days. All of these animals were given as much oats and carrots as they could eat. This is a factor of importance, for we have found that animals fed on carrots only were very much more resistant to mercuric chloride poisoning than were those fed on carrots and oats. We are at present engaged in determining the reason for this interesting fact, and hope to be able to report in the near future our work in this direction. We feel confident, as a result of our

\*From the Pharmacologic Laboratory of the College of Medicine, University of Illinois.

observations, that 0.04 gm. per kg. constitutes a uniformly fatal dose for rabbits, provided the rabbit has been fed on oats and carrots and provided the mercuric chloride solution has been freshly prepared with distilled water; and believe that the results would have been more uniform had a weighed amount of carrots been fed previously, instead of giving the animals all the carrots they would eat in addition to oats.

In the work previously reported, the antidote was given either mixed with or immediately after the administration of the poison. This was, of course, giving the antidote an unfair advantage. For in practice such prompt use of antidote is an impossibility. We, therefore, in the work here to be reported, permitted a uniform interval of 5 minutes to elapse between the administration of the poison and of the antidote, having withdrawn the stomach tube during the interval. It seems that an antidote that displays undoubted efficiency on such a test should be accorded a place in practical therapeutics.

#### CARTER'S ANTIDOTE.

This mixture of sodium phosphite, 10 parts, and of sodium acetate, 6.6 parts, was advocated by Thomas A. Carter<sup>2</sup> on the basis of test tube experiments and of observation of its value in cases of poisoning in the human. Owing to the difficulty of being absolutely certain when life has really been saved in the ordinary conditions of medical practice, owing to the impossibility of accurately comparing the value of different antidotes, and the great practical importance of knowing which antidote is the best, the method employed in our work seems to offer an opportunity for formulating a definite opinion on these questions. By comparing Tables II and III, it will be seen that Carter's Antidote is undoubtedly of value in saving lives of rabbits poisoned with a fatal dose of corrosive mercuric chloride. While no animal in the control series survived 100 days, 7 among 21 rabbits, or 33 $\frac{1}{2}$  per cent, of those treated with Carter's Antidote recovered. The average lethal period in the Carter's Antidote series was 44 $\frac{1}{2}$  days, against 7 $\frac{3}{4}$  days in the control series. This result agrees very well with that obtained in our previous study of this antidote. However, even these figures probably do not represent the real value of the antidote; for, as will be seen from Table III, nearly all the animals that died succumbed within the average fatal period of the control series, as though they had not been given antidote. This suggests that, in these cases, the antidote might not have had a chance to act upon the poison, which might have been deposited in a different part of the food-filled rabbit's stomach from that in which the antidote happened to be introduced. The fact that about half of the number of animals died as though they had had no antidote, while the other half of the series recovered or showed well marked delay in the fatal period suggests that the chances are about even that antidote and poison may mingle with or miss each other in the rabbit's stomach. In a stomach that is empty, like the human stomach after lavage, the chances for antidotal action ought to be decidedly better than in the ever-full rabbit's stomach.

Inasmuch as calomel is formed, when sodium phosphite is mixed with a solution of mercuric chloride, one might expect that sodium phosphite alone would be sufficient for the purpose. This it does not seem to be, as was indicated by the results of our previous study (Table IV).

TABLE II.  
CONTROL TESTS.

ANIMALS FED ON CARROTS AND OATS. DOSE: 0.04 GM. PER KG. CORROSIVE MERCURIC CHLORIDE BY STOMACH.

Rabbit	Weight	HgCl <sub>2</sub> 1%	Lived	Necropsy Lesions	
				Local	General
1	1325	5.3	3	Marked	Marked
2	1448	5.8	6	Slight	Slight
3	1325	5.3	5	Marked	Marked
20	910	3.6	3	"	"
21	905	3.6	2	"	"
22	900	3.6	2	"	"
23	1140	4.5	3	"	"
24	1225	4.9	3	"	"
25	1500	6.0	5	Slight	"
26	1355	5.4	3	"	"
44	1075	5.0	60	Necropsy Lost	
45	1625	6.5	7	Marked	Marked
82	2500	10.0	35	Slight	Slight
83	1400	5.6	4	Marked	Marked
101	1280	5.1	2	"	"
102	1392	5.6	4	"	"
103	1422	5.7	6	"	"
104	1100	4.4	4	"	"
105	1469	5.9	3	"	"
106	1379	5.5	4	"	"
107	1471	5.9	4	"	"
108	1700	6.8	2	"	"

This table shows that the average lethal period for 0.04 gm. of HgCl<sub>2</sub> per kg. is 73½ days. No animal survived 100 days.

TABLE III.

LETHAL DOSE OF MERCURIC CHLORIDE (0.04 GM. PER KG.) FOLLOWED 5 MINUTES LATER BY 10% SOL. OF SODIUM PHOSPHITE (10 TIMES AS MUCH) AND SODIUM ACETATE (6.6 TIMES AS MUCH). (CARTER'S ANTIDOTE.)

Rabbit	Weight	HgCl <sub>2</sub> 1%	Carter's Antidote	Lived	Necropsy Lesions	
					Local	General
4	1465	5.9	5.9	3 days	Marked	Marked
5	1632	6.6	6.6	69 "	Slight	Slight
6	1113	4.4	4.4	7 "	"	"
16	1620	6.4	6.4	100 "	"	"
17	1485	3.9	3.9	100 "	"	"
18	2135	8.5	8.5	12 days	Marked	Marked
58	787	3.1	3.1	4 "	"	"
59	1459	5.8	5.8	5 "	"	"
60	1057	4.2	4.2	100 "	"	"
77	1404	7.6	7.6	100 "	"	"
79	2024	8.0	8.0	100 "	"	"
84	1430	5.7	5.7	100 "	"	"
85	1220	4.8	4.8	40 "	"	"
86	1626	6.5	6.5	100 "	"	"
87	1580	6.3	6.3	2 "	Marked	Marked
88	1471	5.9	5.9	3 "	"	"
89	1411	5.6	5.6	6 "	"	"
92	1351	5.4	5.4	15 "	"	"
96	1675	6.7	6.7	67 "	Negative	Negative
99	1590	6.4	6.4	4 "	Marked	Marked
100	1920	7.7	7.7	3 "	Marked	Marked

Carter's Antidote, when freshly prepared in solution, has a decided antidotal value. Average lethal period 44½ days. Seven animals among 21 (33⅓%) survived 100 days.

TABLE IV.

LETHAL DOSE OF MERCURIC CHLORIDE MIXED WITH (W) OR FOLLOWED BY (F) SODIUM PHOSPHITE.

Rabbit	Weight	Mercuric Chloride		Sodium Phosphite	Lived	Necropsy Lesions	
						Local	General
H	1018	0.068	w	0.68	4 days	0	Marked
H 1	780	0.030	w	0.30	4 "	0	"
HL1	1188	0.047	f	0.47	4 "	Marked	"

Sodium phosphite given alone is of little value as antidote, av. survival 4 days.

Carter believed that the reason for the value of the acetate was to be found in its diuretic action. This can not be the case: for, in the first place, the dose is too small to have much diuretic action; and then, as we have shown in our previous study (Table V), a large excess of acetate, which ought to have a still

TABLE V.

LETHAL DOSE OF MERCURIC CHLORIDE MIXED WITH (W) OR FOLLOWED BY (F) SODIUM PHOSPHITE (10 TIMES AS MUCH) AND SODIUM ACETATE (100 TIMES AS MUCH).

Rabbit	Weight	Mercuric Chloride		Sodium Phosphite	Sodium Acetate	Lived	Necropsy Lesions	
							Local	General
H 4	1749	0.070	w	0.70	7.00	1 day	Slight	Slight
H 6	974	0.039	w	0.39	3.90	3 days	"	"
HL4	1215	0.049	f	0.49	4.90	3 "	Marked	Marked
HL6	1000	0.040	f	0.40	4.00	4 "	Slight	"

Sodium phosphite with large excess of acetate is of no antidotal value.

TABLE VI.

USE OF OLD ANTIDOTAL SOLUTIONS.

Lethal dose of mercuric chloride (0.04 Gm. per Kg.), followed 5 minutes later by antidotal solutions that were kept for varying periods of time. Numbers 31 and 32 were given antidotal solution 1 day old. Carter's Antidote used for the other animals was 2 weeks old. The hypophosphite-hydrogen peroxide solution was 4 weeks old.

Rabbit	Weight	HgCl <sub>2</sub> 1%	Carter's Antidote	Lived	Necropsy Lesions	
					Local	General
32	1560	6.2	6.2	100 days		
111	1532	6.1	6.1	2 "	Marked	Slight
113	1473	5.9	5.9	4 "	"	Marked
116	1853	7.4	7.4	4 "	"	"
118	1210	4.8	4.8	1 day	"	"
121	1153	4.6	4.6	3 days	"	"
			Sod. Hypo-Phosphite 10%	Sol. of Hydrogen Peroxide 1/4 Vol.		
31	980	3.9	3.9	2.0 3 days	Marked	Marked
109	1504	6.0	6.0	1.5 100 "		
122	1093	4.4	4.4	1.1 3 "	Marked	Marked
123	1333	5.3	5.3	1.3 6 "	"	"
125	1331	5.3	5.3	1.3 100 "		
126	1754	7.0	7.0	1.8 1 day	Marked	Marked

It is evident that Carter's Antidote in solution does not keep well, while the keeping qualities in case of the hypophosphite-peroxide mixture are unexpectedly good: for the average lethal period, in the latter case, was 35.5 days; and the percentage of survivals 33%.

greater diuretic action, seems to annul the antidotal value of the phosphite. Both of these series are too small to admit of final conclusions. Nevertheless they suggest that sodium acetate in certain proportions might act by introducing a condition of chemical instability which favors the occurrence of the antidotal reaction. An additional observation points in the same direction (Table VI).

The first half of this table shows that, when a solution of Carter's Antidote is kept for two weeks, it seems to lose its antidotal value. Only the first animal (No. 32), which received as antidote a solution 1 day old, recovered.

There apparently occurs a deterioration of effect on prolonged keeping of this solution, which might be due to rearrangement of ions, with production of a less active complex. Inasmuch as Carter's Antidote may be kept mixed, in the dry state (even in tablet form), this instability of the solution is of little practical consequence.

#### SODIUM HYPOPHOSPHITE AND HYDROGEN PEROXIDE.

This combination was first used, as antidote to mercuric chloride, in our previously published work on this subject.<sup>1</sup> Our reason for using it was the fact that the addition of hydrogen peroxide appreciably accelerated, in test tube experiments, the reduction of mercuric chloride by hypophosphite. That it has antidotal value, even when administered five minutes after the poison, may be seen from Table VII.

TABLE VII.

LETHAL DOSE OF MERCURIC CHLORIDE (0.04 GM. PER KG.) FOLLOWED 5 MINUTES LATER BY SODIUM HYPOPHOSPHITE (10 TIMES AS MUCH) AND VARYING PROPORTIONS OF SOLUTION OF HYDROGEN PEROXIDE.

Rabbit	Weight	HgCl <sub>2</sub> 1%	Sod. Hypo- phosphite 10%	Sol. of Hydrogen Peroxide 1/4 Vol.	Lived	Necropsy Local	Lesions General
10	1065	4.3	4.3	1.1	100 days		
11	1620	6.4	6.4	1.6	77 "	Marked	Marked
12	1700	6.8	6.8	1.7	6 "	Slight	Marked
19	1367	5.5	5.5	1.4	4 "	Marked	Marked
20	1780	7.1	7.1	1.8	4 "	"	"
21	1360	5.4	5.4	1.4	5 "	"	"
61	1568	6.2	6.2	1.5	2 "	"	"
62	1060	4.2	4.2	1.1	100 "		
1/2 Vol.							
7	1330	5.3	5.3	2.7	25 days	Marked	Slight
8	1160	4.6	4.6	2.3	32 "	Slight	"
9	1370	5.4	5.4	2.7	6 "	Marked	"
22	1525	6.1	6.1	3.0	100 "		
23	1030	4.1	4.1	2.0	5 "	Marked	Marked
24	1020	4.0	4.0	2.0	4 "	"	"
= Vol.							
25	1390	5.5	5.5	5.5	31 days	Necropsy	Lost
26	1200	4.8	4.8	4.8	5 "	Marked	Marked
27	970	3.8	3.8	3.8	4 "	"	"
2X Vol.							
28	1490	5.9	5.9	11.8	100 days		
29	1390	5.5	5.5	11.0	4 "	Marked	Marked
30	1395	5.5	5.5	11.0	21 "	"	"
54	945	3.7	3.7	7.4	100 "		
63	986	3.9	3.9	7.8	5 "	Marked	Marked
64	1195	4.8	4.8	9.6	29 "	"	"

Sodium hypophosphite and hydrogen peroxide have antidotal value. Average lethal period 33 1/3 days. Five animals among 23 (22%) survived 100 days.

To determine which proportion is best, we used varying proportions of hydrogen peroxide, with results the significance of which we are at present unable to explain. It will be seen that, when equal volumes of hydrogen peroxide and 10 per cent solution of sodium hypophosphite are used, there is hardly any antidotal action as is shown in the three cases in which it was used. We obtained the same result with two animals in our previous series. The series is, however, still too small to rule out the possibility of accident. With the other proportions, antidotal results were obtained; the best with twice the volume, the next best with one-fourth the volume. Summarizing all the experiments, the hypophosphite combination seems inferior to Carter's Antidote, the percentage of survival being 22 with the hypophosphite combinations against 33 with the phosphite mixture. The average lethal period was lengthened to  $33\frac{1}{3}$  days by hypophosphite against  $44\frac{1}{2}$  days of phosphite antidote. If on the other hand, we take the result of the best proportion, namely with two times the volume of hydrogen peroxide, the results are exactly the same.  $33\frac{1}{3}$  per cent recovery and  $43\frac{1}{6}$  days average fatal period.

It may seem strange that the combination of an oxidizing and a reducing agent should be of advantage in a reducing reaction. A thought that forces itself upon one is the suspicion that the hypophosphite might have been oxidized to phosphite by the hydrogen peroxide, and that the efficiency of the combination depended upon phosphite rather than hypophosphite. That this is not the case, can be shown by means of calcium chloride solution, which precipitates with sodium phosphite—calcium phosphite being insoluble—but which does not precipitate with sodium hypophosphite, owing to solubility of calcium hypophosphite. When we test our previously carefully neutralized antidotal mixture with calcium chloride, we obtain no precipitate, even though the hypophosphite-hydrogen peroxide mixture be previously boiled or be many weeks old. That these two agents do not seem to enter into reaction with each other, on standing, is further borne out by our rather surprising result with old antidotal solutions (Table VI), from which it will be seen that practically the same result was obtained as with fresh solution.

Further experiments are required to elucidate the reason for the efficiency of these combinations. To refer to them as instances of catalytic action would not add much, as we know so little about the real nature of catalysis.

#### CONCLUSIONS.

1. Carter's Antidote has given the best results in this study.
2. The efficiency of a mixture of sodium hypophosphite with hydrogen peroxide is but little inferior, and, in certain proportions, equal to Carter's Antidote.

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## LABORATORY METHODS

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### APPARATUS FOR STUDYING THE EFFECT OF DRUGS ON THE ISOLATED GUINEA PIG UTERUS\*

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THOSE who have occasion to study the effect of drugs on isolated organs suspended in Ringer's or Locke's solution, such as strips of intestine or the various structures from the male and female genital tracts, and particularly those who are engaged in the assay of ergot and pituitary products on the isolated guinea pig uterus, may be interested in a form of apparatus, here described, which has proved very convenient and useful for such purposes.

This apparatus was fashioned somewhat after that described by Dale and Laidlaw,<sup>1</sup> and in its original form, was first set up and used in this laboratory by W. F. Baker. It differs from similar apparatus chiefly in being practically all glass. During the three years or more in which the apparatus has been in almost constant use, several changes and additions have been made that seem to be decided improvements.

In its present form (Fig. 1), the apparatus consists of two units, each unit being complete by itself for the carrying out of an experiment. With the two units an assay of pituitary extract, for example, can be carried out in duplicate, or, two samples can be compared with a standard,—one sample with the standard in one unit, and the other sample with the standard in the other unit. And with this duplicate form of apparatus both horns of a uterus may be utilized,—one in one unit and one in the other.

The essential part of each unit is a water bath with included coils, tubes, etc. The water bath (Fig. 2-1) consists of a bell glass which is inverted and set in a large ring on a heavy ring stand. This bell glass measures 15 cm. wide by 20 cm. deep, inside, and has a capacity of about three liters after the other parts are in place. In its inverted position, the bell glass has one tubulature near the top (3), and one at the bottom (2). The top, or large opening, of the bell glass is covered with a disc of sheet metal—German silver (1), through which pass the various tubes, etc. The tubulatures are closed by rubber stoppers (5 and 5a). The bath is filled with distilled water to a height regulated by an overflow tube (6). Another tube (6a), connected with the overflow tube, allows the bath to be drained.

The water in the bell glass is kept warm by means of a tubular form carbon lamp (7). The size of lamp needed will depend on the room temperature, but 30 watt lamps have been used almost entirely. The lamp can be immersed full length in the bath or only part way, as necessary. When about the right

\*From the Department of Experimental Medicine, Eli Lilly & Company.  
<sup>1</sup>Dale and Laidlaw: Jour. Pharm. and Exper. Therap., 1912-1913, No. 4, 7.

depth is found, the room temperature remaining constant, very little attention is required to keep the temperature of the bath, indicated by thermometer 32, practically constant. In order to maintain an even temperature throughout the bath, the water is kept continually circulating by gently passing through it a current of air which is conveyed to the bottom of the bath by a glass tube (8). In order to exclude the light of the lamp from the uterus or other material used, the lamp is painted black.

Two glass coils are set in the bath, the outer one (9), having a capacity of 200 c.c., is for the purpose of warming the Locke's solution which is allowed to flow into it from an elevated container,—a 2 liter aspirating bottle. By this

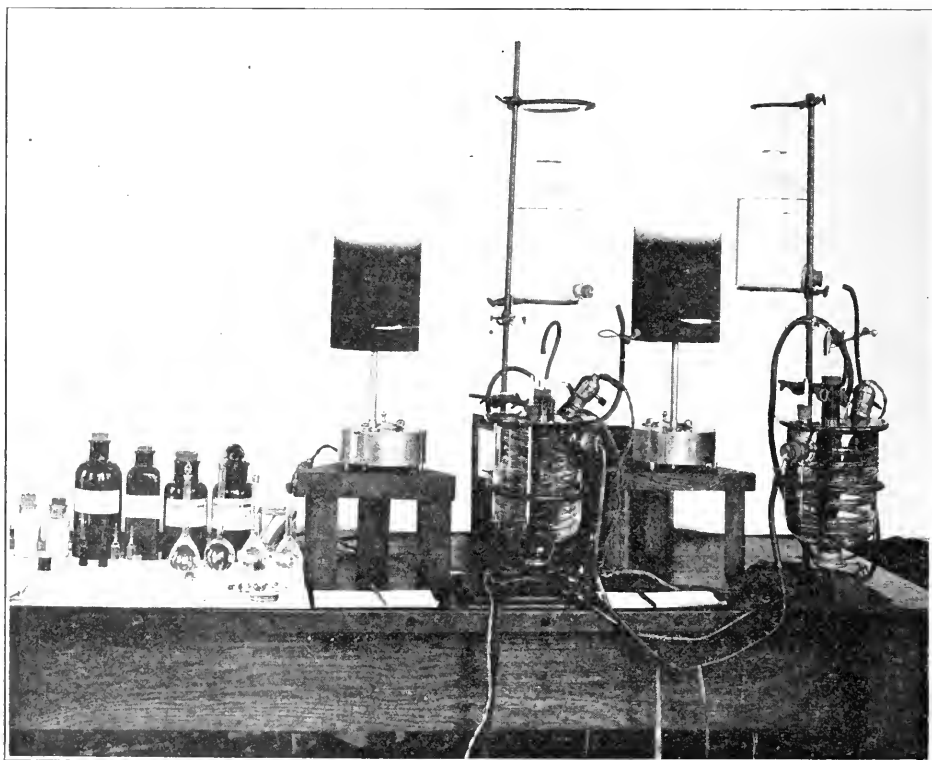


Fig. 1.

method of warming the Locke's solution in small quantities only as needed, precipitation of calcium salt is avoided which would to some extent occur if the whole container of solution were kept warm for some time. The lower end of this coil is joined to a tube forming a T (10), one arm of which (11) passes up through the lower stopper of an amber chamber (14), and the other arm (12) passes down through the stopper at the bottom of the bell glass. By means of a pinchcock at the elevated container (Fig. 1) and the pinchcock 13, the Locke's solution may be flowed down through the coil and either up into the chamber by way of tube 11, or down to a waste bucket by way of tube 12, and, of course, the chamber may be emptied by way of tubes 11 and 12.

The inner coil of the bath (15) is for the purpose of warming the air or



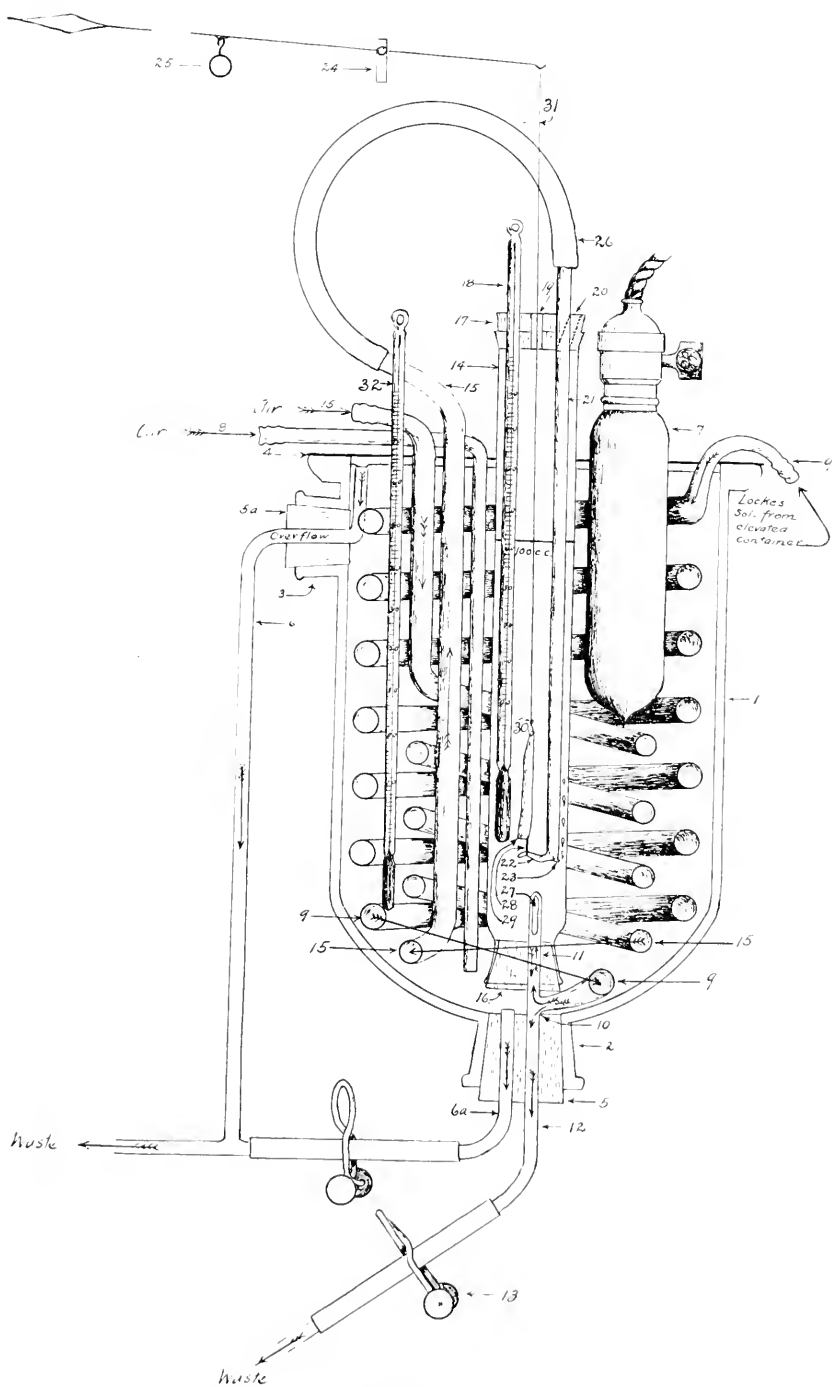


Fig. 2.

oxygen which is to bubble through the Locke's solution in the chamber. The main object of warming the air is to reduce the stimulation which the cool air would give to the uterus in passing about it when the chamber is momentarily emptied. Each time the chamber is emptied, one may stop the air from coming to the apparatus by pinching the rubber air tube, but this is somewhat inconvenient when two pinchcocks, in addition, have to be operated quickly. Managing any way one may, it seems that the emptying of the chamber will surely cause slight stimulation of the uterus, which may result in very slightly increased contractions. During the changing of the solution, however, one may stop the drum and if the writing lever or clamp holding it can be adjusted by a thumb screw, the writing point may be drawn away from the drum, and thus the record will not be disfigured by such added contractions.

The chamber (14) for holding the Locke's solution, in which the uterus or other organ is immersed, is made of amber glass tubing (3 cm. inside diameter) and is graduated to 100 c.c. It bears a cork stopper at the top and a rubber one at the bottom. As previously mentioned, the chamber is filled through tube 11 from coil 9, and is emptied through tubes 11 and 12, the solution flowing off to a waste bucket or drain. Tube 11 has a blind end in the chamber but is slotted on two sides (27) to prevent the current of Locke's solution from striking directly against the uterus when the chamber is filled. The stopper at the top of the chamber holds a thermometer (18) and a tube conveying air or oxygen to the Locke's solution. This stopper also has two holes, one (19) for the thread (31) from the uterus to the writing lever, and another (20) through which the pituitary solution or other drug is introduced.

The introduction of the drug may conveniently be made with a Record syringe having a long needle by means of which the drug can be injected directly into the Locke's solution. The air tube 21 has a small post about 1.5 cm. long near the bottom (22) to which the uterus (29) is attached, and the opposite side of the tube is drawn out into a little point or jet (23) from which the air escapes into the Locke's solution. The tube receives the air from coil 15, which in turn receives it from the tank or compressed air system of the building. Coil 15 is connected with tube 21 by a rubber tube (26).

The thread from the uterus is attached to one arm of a counterbalanced Harvard heart lever (24), the other arm being weighted down (1 to 4 gm.) by hanging upon it small lead weights (25). Harvard slow-speed, single kymographs are used. These instruments are made to turn the drum once in ten hours, but for this purpose are speeded up to about one revolution in eight hours by diminishing the weight of the pendulums.

For the purpose of giving a clear understanding of the operation and use of this apparatus, the testing of pituitary extract will be very briefly outlined. (A somewhat more detailed description of the method is given in another paper.<sup>2</sup>)

#### MATERIALS.

1. Chemically pure salts necessary for making Locke's solution.
2. Distilled water.
3. Pituitary extract to be tested.
4.  $\beta$ -imidazolylethylamine hydrochloride, or standard pituitary substance.
5. The uteri of 175 to 250 gram virgin guinea pigs.

<sup>2</sup>Eckler, Charles R.: *Am. Jour. Pharm.*, 1917, lxxxix, No. 5, 195.

## PROCEDURE.

The distilled water is warmed to 39° C. and introduced into the bell glass. The lamps are turned on and adjusted to the proper depth to maintain the desired temperature. While this adjusting is going on, the Locke's solution may be made up and introduced into the elevated chamber, the dilutions of the extract to be tested and the standard may be made, the drums smoked, the needles

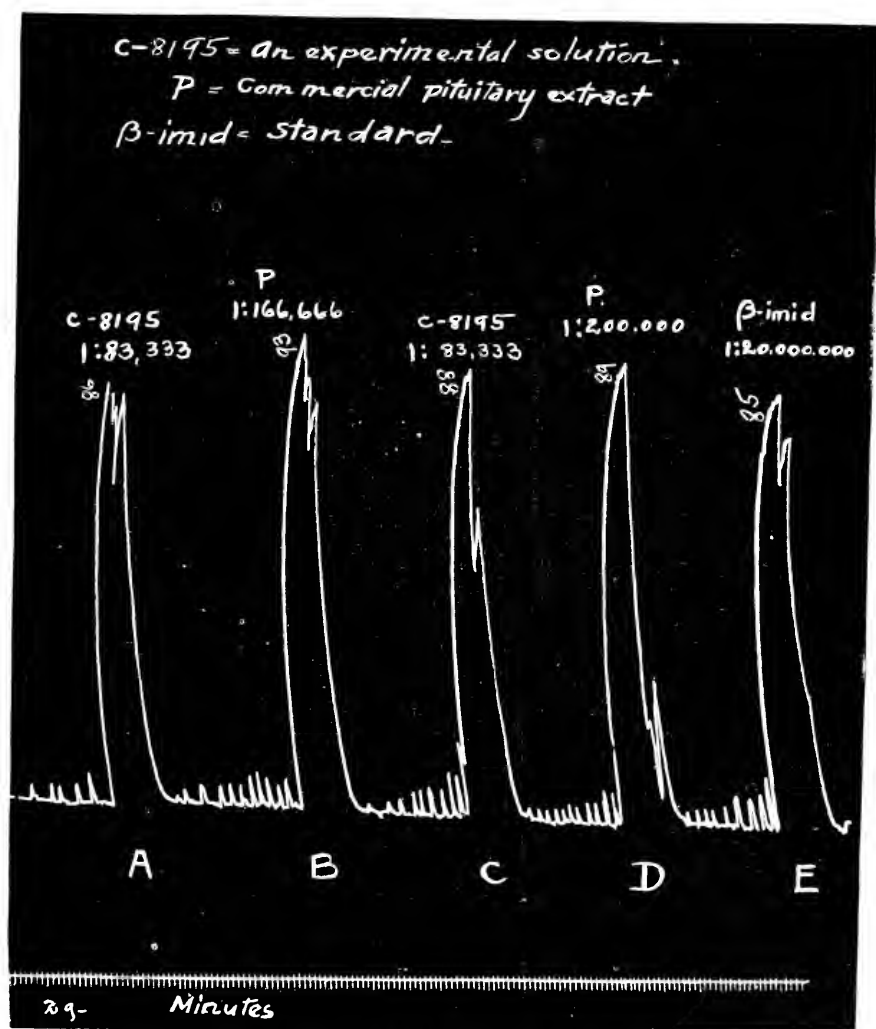


Fig. 3.

threaded, etc. When the apparatus is in readiness, the pig is decapitated, the entire uterus with Fallopian tubes and section of the vagina removed to a fold of cotton saturated with warmed Locke's solution, and the horns of the uterus separated, excluding the section of vagina. The vaginal end of a horn is tied to the little post on the air tube (22) by a silk thread (28) which is sewed into the peritoneal covering on the side of the broad ligament. The ovarian end of

the horn is attached to the thread (31) running to the writing lever by a small pin hook (30) which is passed either through the Fallopian tube or through the peritoneal covering as before.

The writing lever is weighted, and with a thumb-screw adjustment, the writing point is brought to the drum and the drum started. As soon as spontaneous movements of the uterus have appeared, which will usually be in about half an hour, the application of the standard substance is begun. It is first determined whether or not the uterus will react in a quantitative manner, and if so, the standard and unknown are then given alternately until a dilution of each is found which will produce like contractions of the uterus. For example, it may be found that a 1:200,000 dilution of the pituitary extract will cause approximately the same extent of contraction as a 1:20,000,000 dilution of  $\beta$ -imidazolethylamine hydrochloride. (*D* and *E* on kymograph, Fig. 3).

The substances to be tested are carefully measured out with narrow pipettes and introduced into the 100 c.c. chamber with a long-needled Record syringe, in such strength of solution that the final dilution in the chamber will bring the substances to the dilution desired. For example, if it is desired to apply a 1:250,000 dilution of the pituitary extract to the uterus, 0.4 c.c. of a 1:1,000 solution may be introduced into the 100 c.c. chamber and the fluid, which will then measure about 99 c.c., made up to mark by rinsing the syringe and dose cup with sufficient warm Locke's solution, thereby diluting the 1:1,000 dilution 250 times. Or, if it is desired to apply a 1:20,000,000 dilution of  $\beta$ -imid., 0.5 c.c. of a 1:100,000 solution may be introduced into the 100 c.c. chamber and the fluid made up to mark as before, thereby diluting the 1:100,000 solution 200 times. The bubbling of the air through the Locke's solution equally distributes the injected solution in a few seconds.

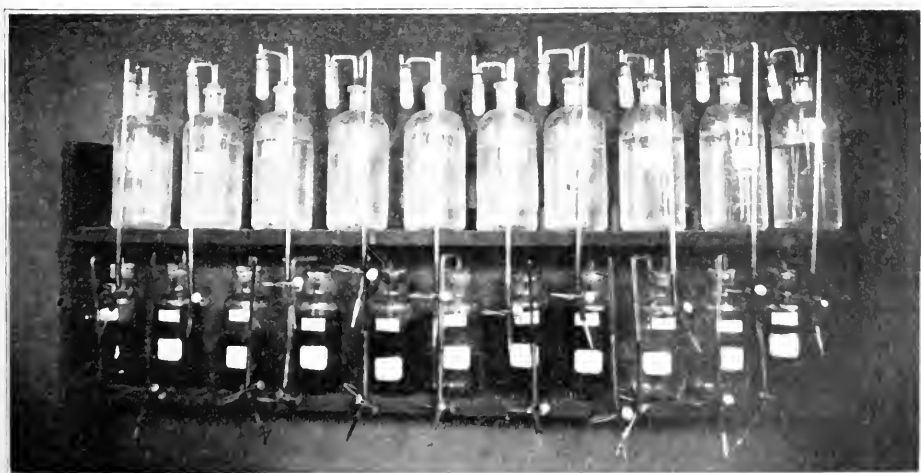
After the application has been made and the writing point has registered the greatest contraction the uterus will make with the given dilution of drug, which will usually require from three to five minutes, the chamber is emptied by opening the pinchcock 13, when the fluid will flow out through tubes 11 and 12. Closing this pinchcock and opening the one at the elevated container, Locke's solution will flow in from coil 9 and fill the chamber. In order to remove all considerable traces of the drug which may have adhered to the sides of the chamber or diffused from the wall of the uterus, the chamber is emptied again and refilled to within 1 or 2 c.c. of the 100 c.c. mark. After about 15 to 30 minutes, depending on the activity of the particular uterus, another application of drug is made, and so on, until the end result is reached.

# THE USE OF INDICATORS TO DETERMINE H-ION CONCENTRATION—APPARATUS FOR TEACHING\*

BY LOUIS ROSENBERG, B.A., DALLAS, TEXAS.

WITH very little modification the following introduction from Professor Carmichael's address on Mathematics in the May 18th issue of *Science* applies to chemistry in the medical curriculum: "Mathematics beyond the merest elements has been regarded by some as an excrescent malady by the human spirit, generated like the pearl in an abnormal and morbid way and representing a nonliving embedment in the active tissue of the organism of society; by others it has been supposed to exhibit the highest intellectual reach of mankind, being in itself the most powerful tool yet devised for the interpretation of natural phenomena, while at the same time it affords a satisfying expression of the furthestmost esthetic attainment. On the one hand, it is considered a piece of jugglery in which it is the joy of the proficient to produce more and more complicated entanglements to astonish the beholder and overwhelm him with the sense of mystery; on the other hand, it is seen to be the systematic unfolding of remarkable and important properties of a highly fascinating creation or construction of the human spirit by means of which it has at once its most intellectual delight and the best means of understanding its environment. . . . But it is true, I believe, that mathematics is generally recognized as essential at least to scientific progress."

I take it therefore that those who are striving to teach the chemistry that modern medical students should learn have met the same difficulties as the writer: woefully deficient preliminary training in the science, absolute lack of interest, forced and inefficient application and plenty of charitable advice by well-meaning but ill-advised practitioners that *their* chemical courses were a



\*From the Department of Chemistry, Baylor University Medical College, Dallas, Texas.

joke, that they never used chemistry in their practice and that it is more than a nuisance in the medical curriculum.

I believe, therefore, that you would welcome any suggestion that will facilitate the teaching of any concept that we believe of fundamental value in the equipment of the modern medical student and I submit herewith a device designed and made by the writer which has proved invaluable in the teaching of the subject of indicators and their use to determine H-ion concentration.

Referring to the cut it is seen that the apparatus consists essentially of a portable rack having two shelves: the upper carries one liter bottles containing solutions of H-ion concentrations unknown to the student; the lower shelf carries the indicators arranged in a series of descending H-ion reaction. The solutions are prepared according to the chart on page 157 of Hawk's Practical Physiological Chemistry; the indicators are selected from the lists in the above text and in Mathews' Physiological Chemistry. I have found the following series of indicators best: Nauvein, Tropaeolin 00, Methyl Orange, Methyl Red, Congo Red, p-Nitro-Phenol, Rosolic Acid, Tropaeolin 000, Phenolphthalein, Thymolphthalein and Tropaeolin 0.

The general design and arrangement are clearly shown in the cut. The only feature requiring comment perhaps is that by which evaporation of the solutions in the top row of bottles is prevented. At first a mercury valve was employed but this proved unsatisfactory. The arrangement shown can be made very easily by sealing off small test tubes to the desired length and using small tubes drawn out to about a one millimeter capillary, thus to expose a minimal area of the solution in the test tube, which is of course the same as in the associated bottle.

The apparatus is inexpensive and can be made up, solutions and all, in a few days.

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## MASS URINALYSIS

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BY MORTIMER WARREN, M.D., NEW YORK CITY.

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THE knowledge of the essential value of laboratory examinations, in diagnosis, is universal.

Independent laboratories have come into existence to furnish one of the means by which the physician can meet this demand of his practice. The laboratory has, in fact, assumed the responsibility for the performance of even those simple tests which compose a routine analysis of urine. The concentration of effort resulting from the separation of duties should lead to increased reliability of method and should furnish accurate information within the limits of the service undertaken by the laboratory.

The laboratory, however, tends to become completely disassociated from the stimulus of the problems of diagnosis. A laboratory thus constructed is, in essence of organization, a factory. It follows that the bulk of the work will be done by a technically trained staff whose labor in great part consists of monotonous repetition of routine procedures.

The system in vogue in life insurance laboratories in general emphasizes the specimen of urine as the unit. Here the object is to classify a given individual on the basis of the urinary findings. Casts, for example, are considered to be of so much significance that an exhaustive search of the sediment is required.

Another system which has certain advantages, when a large number of examinations are to be carried on, makes use of the individual test as the unit. So far as sediment is concerned, an average time limit can be found which will broadly cover the ground. The expression "Casts not found" then means that no casts were found in the time devoted to the search. My remarks are concerned with a personal experience in a laboratory which has conducted its work along the lines mentioned above. It has endeavored to standardize the methods and has subjected all specimens to the same routine with additions to suit indications. I believe it may be of interest to outline the methods in use and to discuss briefly the results obtained so far as they are accessible. The system is a composite which would naturally be developed in some form by anyone under similar conditions.

#### ROUTINE.

1. Color.
2. Reaction (litmus paper).
3. Specific gravity (room temperature).
4. Albumin and quantity if in sufficient amount.
5. Sugar and quantity if in sufficient amount.
6. Indican (when requested).
7. Bile pigments
8. Blood pigments
9. Urobilin (if color gives indication, or if requested).
10. Acetone bodies (ferric chloride test) (if requested or when sugar is present).
11. Urea (hypobromite method) (if requested).
12. Sediment.
  1. Epithelium—flat—round (small or large).
  2. Leucocytes—relative abundance or absence.
  3. Erythrocytes—relative abundance or absence.
  4. Crystals—character.
  5. Casts—relative abundance or absence—character.
  6. Bacteria—yeast, etc., and other elements.

#### PROCEDURE OF EXAMINATION.

Specimens are entered on successively numbered cards which follow the specimens as they are examined. Special requests or remarks are noted. The specimens are run through in sets of a size which varies according to daily circumstance. The bottles are arranged and numbered 1, 2, 3, etc., from left to right with a grease pencil, the accompanying cards being numbered to correspond. Other sets of the same day are numbered 1', 2', 3', etc.

#### STEPS.—

A. Specific gravity—appearance—color—reaction.

B. The bottles are well shaken and the urine is poured into 15 c.c. centrifuge tubes in a rack of two rows, holding twenty-four in all. These tubes are

numbered 1, 2, 3, etc., from left to right. They are centrifugated for a specified time at a standard speed.

C. From the centrifugated tubes the supernatant fluid is carefully decanted from the visible sediment into test tubes in a rack for albumin, sugar, etc. The sediment in the centrifuge tubes is reserved for microscopic examination.

D. From rack C, always left to right, (1) a rack of 24 tubes is filled one-half full of urine for albumin; (2) to the 24 tubes of a copper rack, each of which contains Benedict's solution up to a certain mark (cross beam of rack), is added about one-sixth volume of each one of the specimens from rack C; (3) remainder of fluid in rack C is reserved for other examinations.

METHODS.—*Albumin*.—To each test tube of D<sup>1</sup> is added one-sixth volume of saturated sodium chloride solution (NaCl<sub>2</sub>) C. P. and two or three drops of 3 per cent acetic acid. The upper portion is heated to boiling in a rack devised for the purpose, two or three drops of acetic acid are added and the upper layer is examined by means of a bull's-eye light against a black background. Presence of albumin is noted as +, ++, +++, and more than +++ is determined quantitatively by Purdy's centrifugal method.

*Treatment of Cloudy Urine*.—To a test tube one-half full of urine is added 1 c.cm. of 10 per cent sodium hydrate; the urine is filtered,\* the filtrate is acidified, and the usual albumin test is proceeded with as above.

*Sugar*.—Rack (D<sub>2</sub>) is immersed in a boiling calcium chloride bath for two minutes (calcium chloride, technical—saturated watery solution) (Myers Hospital urinalysis). Those tubes which show reduction are noted and the finding is confirmed by a second test with Haines solution. In case of difficulty of interpretation, a phenylhydrazine test is done.

*Quantitative Sugar (Adaptation of Benedict's Method)*.—Five c.c. of Benedict's reagent in a porcelain dish with one-half teaspoonful of calcium carbonate is placed over a flame. The urine is run in from a 10 c.c. Mohr pipette (urine diluted 1 to 10). A table for readings is constructed which gives twenty-one readings from 0.2 per cent to 10 per cent, varying in the lowest range by 0.2 per cent, in the medium by 0.5 per cent, and in the highest by 1 per cent. In this procedure it is taken for granted that every reducing substance which gives a positive phenylhydrazine reaction or which allows of a definite titration with Benedict's quantitative reagent is glucose. No attempt is made to separate the unusual carbohydrates.

*Sediment*.—Tubes of rack B are kept in this rack until examined. From each tube the sediment is carefully decanted so as to leave about ½ c.c. of residue. This is thoroughly shaken and deposited on a glass slide by tapping the upturned tube against the slide. An examination is made with low power of the entire drop; undifferentiated elements, if present, are examined with high power.

Aside from indican, other methods outlined in the routine require no ex-

\*Centrifugation clears urine as well as filtration does. A cloud composed of urates dissolves with heat, one due to phosphates with acid. A cloud composed of bacteria will not be removed by filtration or by centrifugation; in this case the sodium hydrate method of clarification is used. Occasionally a trace of albumin will develop in these urines when not originally present, due apparently to bacterial proteins. The practical result of this procedure appears to be fairly reliable although the precipitation possibilities of albumin may be interfered with by the addition of alkali. (I am unaware of the source of this method.)



planation. The method in use for indican is Jaffe's, which consists of clearing urine with lead acetate, adding equal amounts of Obermeyer's reagent and 1 c.c. of chloroform. The test is time-consuming owing to the slowness of the reaction and thus the necessity for repeated periods of agitation and sedimentation. The mere presence of indican can hardly be called abnormal. Its determination should be supplemented by an estimation of the amount present. This is done by noting the number of drops of potassium chlorate solution (3.2 per cent aqueous) necessary to decolorize the indigo. The findings in certain urines may then have interpretative value though the specimen of urine is a single one.

#### DISCUSSION.

In order to arrive at any conclusive opinion of the value of such mass urinary examinations, it is necessary to consider what is to be expected:

1. The work should be reliable. Every notation should represent the result of definite procedures.

2. Abnormal elements should be detected and certain of them expressed quantitatively.

3. Expression of findings should be simple and direct. There should be no attempt to distort relative values or emphasize the importance of the work by means of estimations and calculations of a purely paper significance.

4. It should be understood that no evidence of a metabolic nature can be obtained from quantitative estimations of single specimens of urine. No specific diagnosis is possible except in rare instances such as Bence-Jones' proteinuria.

1. *Reliability of Work thus Conducted.*—In general, the character of the work is a direct reflection on the character of supervision. An intelligent, efficient, and conscientious staff is comparatively easy to assemble. Supervision covers outline of methods, distribution of labor, development of *esprit de corps* and sympathetic knowledge of the character of the work and of the relation of fatigue to objective accuracy.

This system, I believe, provides all one could ask in furnishing the information which may be derived from routine examinations. Its actual product is difficult to analyze. In an attempt to ascertain the character of results, specimens were sent through at various times in duplicate. In the series there was in one instance a variation of specific gravity of 3 points, failure to find a few hyaline and granular casts in another instance, a variation of 4 per cent in bulk albumin percentage in another. Otherwise, the findings were in agreement. To determine the personal element in albumin readings a series of forty-eight urines were read by five different individuals. In this series the maximum number of specimens without albumin was, according to one observer, 36, to another, 24. On the other hand, there was entire agreement on large amounts with shades of difference between so-called ++ and ++++. In other words, it is obvious that the shading of the nonalbuminous urine into that containing more or less definite amounts is such that absolute agreement can not be hoped for. In fact, the reported presence of a faint trace of albumin without confirmation or without other evidence on the clinical side has no significance. The finding of albumin or casts or both can not be interpreted by the laboratory.

Their importance (in case the finding is accidental) is simply to draw the closer attention of the physician to his patient.

2. *The Detection of Abnormal Elements*.—Bile, blood pigments, urobilin and other coloring matters would not be found unless in sufficient amount to attract the attention of the observer who notes the color, appearance, etc. Whether Bence-Jones' protein would be discovered or not I can not say by actual experience. In the only case which I have seen, this was overlooked in the routine examination of urine in a hospital. The routine method here outlined, using a rack and heating several urines at a time, would, I believe, offer less chance for the error than the hurried individual test where one, unless expectant, is likely, owing to the early precipitation of the protein, to shorten the necessary period of actual boiling.

Paragraphs 3 and 4 are obvious statements.

#### CONCLUSION.

I believe this system fairly meets the requirements, allowing for a small percentage of error. I do, however, believe it should develop greater accuracy. It lacks the element of confirmation. This can readily be obtained by duplicate examinations in the case of description, specific gravity, albumin and sugar. The two latter tests should be repeated for exactly the same reason that no one depends upon a Wassermann reaction which relies on a single test and its control. Though the importance is less, the principle is the same. An approximately accurate determination of the specific gravity is important from a functional point of view when the fluid intake of the patient is under control. There is special liability in "piece work" to read the urinometer before it has come to rest under proper conditions.

The description of urine, its color and appearance have an important bearing on the subsequent examination and therefore can not be too carefully noted. In conducting these confirmatory tests, a second portion of centrifuged urine would be furnished which would allow of a second examination in cases where there is reason to believe the particular drop under observation is not representative.

The collection and preservation of urine are important factors. Under the conditions which I have described, in the summer months a large proportion of the urines examined are so contaminated that the microscopic examination is of no value and represents a lot of useless labor. Urine should be properly collected. It should, so far as is practicable, represent the kidneys, bladder, and urethra, and not the prepuce and vagina. The urine should be collected in a scrupulously cleaned vessel and immediately delivered into a sterilized bottle, or where possible, passed into the bottle, after thorough cleansing of the parts. Preservation of urine is unsatisfactory in my experience. Boric acid, which is the only practicable substance on account of its noninterference with the usual tests, is only mildly antiputrefactive; but I believe it would have a much better chance if the above precautions were used in collection.

Centrifuge tubes should be cleaned with soap and water, then with weak acid and thoroughly rinsed, drained, dried, and sterilized with hot air. All test tubes should be put through the same process; there is, however, no need

of sterilization. Centrifuge tubes require sterilization in order to avoid as much as is possible the rapid deterioration of urine which takes place in the moist air of the laboratory in the period which may elapse between centrifugation and examination.

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## GUINEA PIGS FOR PROFIT

BY M. A. KROEBER, MT. VERNON, N. Y.

ONE finds it easy to do what one can do well. This applies to raising guinea pigs as to other things; and it is easy to do it well for guinea pigs because they give satisfactory returns for simple care. With proper food at regular intervals and clean cages the caretaker does not need to know much about causes of death or treatment of disease.

The guinea pig's only enemies, if kept in the house, are rats, but outdoor runs must be protected from cats and dogs.

To have recognition in supplying hospitals with animals the breeder must be able to raise them in large numbers so as to continually reserve a sufficient number from which to breed so that the stock may not diminish toward spring. The laboratories make small, practically no demands for animals during the summer months, so that in September the supply is in excess of the demand. By February, however, there are not so many on the market, and by May there is a shortage among the dealers. This causes a temptation for the breeder to sell more in March and the following months than is consistent with reserving stock for the fall.

Some hospitals use animals as small as ten ounces, others stipulate at least five hundred grammes. To raise an animal to the latter weight consumes six months. All circumstances being favorable, they may reach that condition somewhat before, but adverse circumstances may easily prevent. Among the causes which deter the rapid attainment of weight is premature conception, animals not being in a condition for mating under six months of age and eight months is distinctly advantageous, for a mature mother shows no ill effects from bearing young and makes a rapid resumption of strength for another healthy litter of large animals. If mature animals are used to breed from, there are no abortions and full litters of well-developed young are produced. When mothers are too young the first litter may cause the death of the mother, or though the first may be healthy, subsequent litters are usually spindly and inclined to still-births. First litters do not number as many animals at later litters, one or two being usual. The mother thereafter produces three to five, more being out of the ordinary and undesirable from the standpoint of vigor.

In order to prevent animals from conceiving prematurely, the sexes must be separated at the age of one month, when the young are taken from the mother. A number of small animals may be kept to advantage in the same cage if of an approximate age. If large and small animals are kept together the weaker are trampled upon and are not allowed sufficient food. As the animals approach six

months of age the males develop decidedly pugilistic tendencies which makes it desirable to sell them early, keeping only necessary mates. The females are not prone to fight until after successive families when they lose their smoothness of temper. It is best to have only two mothers in a cage together at the time of birth and nursing.

Six or even eight females can be allowed to a male at a time, but they do better if they are separated from the mate when in kindle about a month. They carry their young two months and nurse them from three to four weeks. Four litters, therefore, is the most one can raise from a female in a year, a good mother often having only three—ten to twelve babies a year in all. A good breeder in her prime produces twenty-two or twenty-four young with neither still-birth nor abortion. She should not breed after three years of age, and three years is the outside limit of desirability for sale. With good care they do live three or four years longer as pets.

Oats and hay with abundant green food keep guinea pigs in the pink of condition, the succulent food being a laxative without which they do not thrive, while if the oats are omitted they fail in attaining good weight in short time. Bread is eaten by some but is not economical; neither is grass advantageous in the diet if one aims to get rapid results.

A healthy animal is sleek of fur, clear of eye, and cheerful—moping being a sure sign of sickness. The animals thrive best in a temperature not under fifty degrees, which puts draughty barns out of the question as quarters in winter. They can stand a cool habitation but if exposed to draught or wet bedding succumb to pneumonia.

Sawdust or hay make the best bedding, preferably the former, and an insecticide is never needed if the cracks and corners of the cages are kept clean.

If an animal is ill from cold or injudicious feeding two or three drops of castor-oil can be given to advantage and the dose repeated if necessary. For loose bowels omit the green food for two or three days or for constipation supply greens in abundance—clover, dandelion, lettuce, celery, beet-tops, carrots or carrot tops, etc.

The floor of cages should be impervious to moisture, either of zinc, galvanized iron, or of wood treated with a moisture-, acid-, alkali-proof paint. Ventilation should be good, fresh air being absolutely necessary. An escape of illuminating gas is fatal.

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## EDITORIALS

### *The Physician's Responsibility in Connection With the Food Situation*

**E**VEN prior to the present unusual economic conditions created by the war for democracy, cries of the increased cost of living were heard on every side. Much of this increase, particularly in the case of food, has been due not so much to any real scarcity in the staples of life as to the development of more luxurious habits of living. These concern not alone the particular food-stuffs, but the manner of their delivery to the consumer; elaborate retail stores in expensive buildings, the use of fancy and unnecessary packages and containers, and insistence on prompt delivery, have all contributed toward raising the retail price of food far above its real value. The trouble with us has been not so much the increased cost of living as the cost of increased ease and elegance of living. The wealth of the country has been so great that the consequences of extravagance were not felt by the people as a whole; but now we face the possibility of a real shortage of food—at least what would be one were the standards of the past few years insisted upon. To avoid such a catastrophe we must recast our ways of living; we must learn how to secure an ample

sufficiency of nutriment for even the poorest of our people from other sources of foodstuffs than those that we have been accustomed to use. No one who is familiar with the actual state of affairs has even hinted at the possibility of an insufficiency of nourishment for every individual in this country, provided there is no waste and the dietaries are so adjusted as to use those foodstuffs that are available. There is no danger of a food shortage if the available supply is properly used, but such might easily develop and a state of partial famine become established, did we continue to live by our old wasteful standards.

The greater part of this adjustment can be made by centralized administrative power, but this alone may prove inadequate, and the administration be seriously embarrassed in its work by popular disapproval and misunderstanding, unless the actual consumer comprehends the principles upon which such control must, and no doubt will be, based.

Elsewhere in this Journal is published an article dealing in an elementary way with the principles upon which control of diet is based. It is thoroughly appreciated that to the majority of the readers of the Journal many parts of such an article will appear to be superfluous, because they deal with elementary truths of dietetic physiology which every physician knows or used to know, but it is hoped that placing the newer less familiar pieces of knowledge in their proper relationship with the old will be considered as sufficient justification for adopting such a method.

In the readjustment of diet which each family must probably make during the ensuing year, it will frequently be necessary for the physician to see that no serious mistakes leading to under-nutrition have been made. He will no doubt be consulted by his patients on this particular point, and it can not but help him in giving good advice if he avails himself of an opportunity to rub off some of the rust which from disuse must have developed on the machine of dietetic physiology and hygiene which as a student he was more or less familiar with. The old machine has, moreover, become much changed and added to in recent years.

The average diet used by the individuals of a community depends on a variety of circumstances. For practical purposes these may be divided into two groups, of which the one—the economic—concerns the easiest, most convenient way of supplying the food, and the second—the physiological or hygienic or personal—concerns the requirements of the body for food. It is particularly with the second of these that the housewife can be of incalculable service if she places herself in a position rightly to adjust the diet so as not to interfere with its food value. If the economic situation should make it necessary that changes be made in the amount and nature of the food allowed each individual, it will rest with the housewife to adjust her table to the new requirements without interfering with the efficiency of the diet. In order to do this she must have some means for measuring the adequacy of food—some value or standard by which she may appraise its true nutritive worth. If, for example, the available supplies should demand that each individual must replace in his diet some foodstuff, such as wheat flour, to the use of which he has become accustomed, with other less familiar foodstuffs, how are we to know how much of the one should be used to replace the other? Unless the substitution be in-

telligently made, serious disturbances to health are very likely to follow, especially in the case of the young and growing members of the household.

In the present conflict between autocracy and democracy in which our country is engaged, the one thing upon which victory will rest is efficiency—efficiency not alone of those who are fighting for us on the battlefields and of the administrative authorities of our army and navy, but efficiency of the people at home. In no way can this domestic efficiency with regard to the problem of the adequate and intelligent household administration of food display more practical effect than now at the very outset of this country's entry into the war. By carrying scientific efficiency into every detail of our living, we can beat the Germans at their own game.

The object of the present article is to explain the standards upon which this control of diet depends. It is not intended to prescribe in detail what foodstuffs should go to make up a particular dietary, for this must vary with the requirements, the individual tastes, and the purse of the family, but to explain in simple language the principles upon which such details can be worked out. To present you with innumerable diet sheets for every conceivable condition that might arise, would not only be confusing, but as useless in instructing one in the principles of dietetics as it would be to insist on a medical student's learning by heart the composition of a medicine for each disease he might meet with in his practice. We do not attempt to do so, but we make him study the principles upon which he may himself compose his medicine to suit the peculiar symptoms of each particular case.

—J. J. R. M.

### *The Occurrence of a Lymphocytosis as One of the Late Symptoms of Gas Poisoning*

A PECULIAR and striking change in the lymphocyte cell count of the blood in patients who have suffered from gas poisoning has recently been observed by Miller<sup>1</sup> and Rainy.<sup>2</sup> This change consists in a very marked increase, both relative and absolute, in the number of lymphocytes. It would appear that this increase develops only after a period of a month or six weeks following severe gas poisoning, or after a somewhat longer period if the intoxication was of a milder degree. And in cases with persistent symptoms the lymphocytosis may continue a long time, at least for eighteen months.

The type of cells which is increased is the small lymphocyte with relatively large deeply staining nucleus and relatively little protoplasm. In a few cases there was a fair sprinkling of larger lymphocytes with a broader rim of protoplasm and a larger nucleus. No other type of blood cell appeared to be influenced in any constant fashion.

In a series of normal persons the average number of polymorphs was about 70 per cent, while the lymphocytes constituted about 20 per cent of the total white cell count. This was found in cases in which the total leucocyte count

<sup>1</sup>Miller, Capt. James, and Rainy, Capt. Harry: *The Lancet*, London, 1917, excv, Jan. 6, 19.

<sup>2</sup>Miller, Capt. James: *Ibid.*, 1917, excii, May 26, 793.

varied from 5500 to 9000 per cubic millimeter. But in marked cases of gas poisoning it was found that when a general leucocytosis occurred there was also an absolute increase in the number of lymphocytes; for example, in one severe case with a total white cell count of 17,000 per cubic millimeter there were 6401 (37%) polymorphs and 10,034 (58%) of lymphocytes. Apparently in a relatively very small number of cases (usually mild ones) this phenomenon was scarcely noticeable, the number of lymphocytes being almost, if not quite, down to the normal limits. But its constancy in the great majority of cases in which symptoms persist is regarded as of particular significance, especially in the matter of diagnosis and in the estimation of the degree of severity of the injury produced by a previous exposure to a poisonous gas.

The question of the character of the gas which may produce these effects arises at once. On this point the information obtained was unfortunately very indefinite. The blood change would appear to have no relation to the nature of the gas employed. Undoubtedly some of the cases were due to chlorine, but others were evidently due to other forms of gas, and the conclusion is drawn by the observers that probably all the poisonous gases which have so far been extensively used in the war may produce this effect. On the basis of the late symptoms the cases could be roughly classified into three groups, viz., those in which gastric symptoms predominated, those in which respiratory disorders were more prominent (not infrequently both types were met with together), and finally cases which exhibited chiefly nervous symptoms. But apparently the character and origin of these symptoms in no wise influenced the extent and nature of the blood changes.

Lymphocytosis is a characteristic symptom in a considerable variety of pathological conditions. The authors have considered those conditions carefully in order to determine whether or not they could find any common feature or lesion which might also be present in patients suffering from the late effects of gas poisoning, and which might throw some light on the origin of the lymphocytosis. These considerations have led to the tentative conclusion that probably a chronic inflammatory change in the respiratory and gastric mucus membranes is at least a factor in the production of the blood changes.

It is further significant to note that after a long and careful study of many cases of gas poisoning the authors find it necessary to state, "we are still in the early stages of our knowledge of the effects of gas poisoning on the body."

—D. E. J.



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## *ORIGINAL ARTICLES*

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### OPERATIVE TREATMENT OF PERIPHERAL NERVES AFTER SEVERANCE, MORE PARTICULARLY AFTER LOSS OF SUBSTANCE—A CRITICAL REVIEW\*

BY G. CARL HUBER, M.D., ANN ARBOR, MICH.

ALL available contributions relative to injuries of peripheral nerves in recent wars, and particularly the present war, call attention to the relatively large number of such injuries. This is attributed in part as due to the character of modern small and large arms and to a large extent to the success of modern surgery, by means of which the necessity for amputations is obviated and indeed life itself maintained. Available accounts of the Russo-Japanese War, the first and second Balkan Wars, to a lesser extent the Boer War, bring these facts to light; more particularly is this true of the present war. Bernhardt, writing early in 1915, states that of the approximately five hundred thousand wounded of the Central Powers, seven to eight thousand had received injuries to peripheral nerves. Tinel, who has made use of the wealth of material collected by the Neurological Society of Paris, estimates that 18 to 20 per cent of the men wounded in the limbs have more or less important injuries to peripheral nerves and the percentages are higher if cases of slight trauma are eliminated. Tinel estimates that 60 to 70 per cent of the cases recover spontaneously, although in 10 to 20 per cent of these, surgical intervention would have quickened the process of cure. Surgical operation for the suture of a divided peripheral nerve or for its liberation from grip of cicatricial tissue is required in 30 to 40 per cent. The results of suture should be satisfactory in 85 to 88 per cent of cases operated upon, better if suture is applied early. (I regret to say that I have not been able to obtain this valuable work of Tinel. I am dependent on reviews in British literature.)

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Since injuries to peripheral nerves form so large a percentage of the war injuries, and since the results of such injuries are of such serious nature, all light which can be thrown on their probable relief and cure can not help but be welcomed. Cognizant of this, the writer of this report, who is not a surgeon and is wholly lacking in surgical and clinical experience, feels that he is justified in presenting observation made in laboratory experiments, especially with reference to repair of nerves after loss of substance, in the hope that some considerations of value may be presented. This report is made then, from the viewpoint of the laboratory worker, and the clinical observations cited are interpreted from this viewpoint and not from that of the clinician.

As is well known, the peripheral portion of a severed nerve degenerates from seat of injury to its termination, whether immediately sutured or not. The central stump degenerates to the extent of 1 cm. to 1.5 cm. The details of the process of degeneration need not be given here. Suffice it to say that three or four days after the injury, the myelin sheaths and neuraxes fragment and there is observed the beginning of an active proliferation of the sheath cells. During the following ten to fifteen days, the myelin and neuraxis segments are largely absorbed while the proliferating sheath cells with their protoplasm unite to form syncytial bands or strands of multinucleated protoplasm found in the old neurolemma sheaths. These strands—"Bandfasern"—persist for many weeks even though the peripheral end is completely severed from its central portion. These "Bandfasern" do not conduct nerve impulses and have no demonstrable trophic influence on the tissues innervated by the respective nerve prior to severance.

Regeneration of the peripheral, degenerated portion is from the central uninjured stump. Numerous, five to ten and even more, embryonic neuraxes have been observed to sprout from a single central neuraxis and to grow toward the severed end, and if mechanical conditions are such as to admit of it, reach the peripheral end, to grow down in, and perhaps between, the old sheaths of the degenerated portion, ultimately to reach the periphery. Active outgrowth of the central neuraxes begins about the fifteenth to twentieth day after severance of the nerve. The rate of growth is estimated to be about 1 mm. to 2 mm. a day, in favorable cases; a fact to be borne in mind in judging of the results of operations for repair of peripheral nerves. I believe I am justified in stating that the results of all the more recent work on nerve regeneration indicate a monophyletic origin of regenerating neuraxes, namely, from the central uninjured stump, the peripheral, degenerated portion taking no active part in this process.

Abundant experimental and clinical results are at hand warranting expectation of favorable results after suture of severed peripheral nerves, neurotization of the peripheral portion taking place in the great majority of cases. This is a practice so well known in surgery that stress need not be given it here. Experimental and clinical observations go to show that the results are more favorable the sooner after nerve severance the suture is applied and the more carefully and accurately the divided ends are sutured, and the nearer the periphery the injury occurs. In civil practice, the suture of nerves, either primary or secondary, is usually not attended with unusual difficulties, judging from reports. In recent wars, more especially in the present war, the results of the modern bullet and of artillery fire, etc., are of such a nature that the injuries to nerves are much more difficult of treatment. Nearly all of the articles I have had op-

portunity to read call attention to the difficulty and often impossibility of giving the necessary care to injuries of peripheral nerves at the front, owing to the character of the wounds, in many cases, or for other reasons. The great majority of the cases reported, therefore, come from base hospitals and are treated weeks and often months after the receipt of the injury. In many cases reported the severed ends of nerves were so extensively involved in dense and extensive, often callous-like cicatrices, that resection of the nerve ends to the extent of several centimeters is necessitated, before the nerve ends can be freed and normal nerve tissue is reached. Even with nerve trunks only partially cut or the larger nerve trunks pierced by a rifle-bullet, both of which injuries seem not uncommon in the present war, the cicatricial tissue developing secondarily at the seat of injury is such as to produce, owing to contraction, either severe neuralgic pains or partial or complete destruction of the nerve involved, necessitating neurolysis or resection of the nerve. Operative repair of nerves after loss of nerve substance has received especial consideration in civil practice. Abundant consideration has been given this question in recent and the present wars, and it is particularly to this phase of the question that I desire to draw attention, and again, primarily, from the viewpoint of the laboratory worker. In the course of this report, citations from the literature covering the earlier period of this war, are made. In the great majority of the cases reported, these were reported before sufficient time had elapsed to determine the ultimate result of the operative procedure used. The German literature, both surgical and neurologic, covering the period of the war to the time German publications ceased to be received, teems with reports of war injuries to peripheral nerves. The French and especially the English literature is not nearly so rich in such reports, the articles consisting largely of briefer accounts of small groups of cases or isolated cases found in society transactions and journals. The work of Tinel, French, very probably of great value, I have not been able to see in the original, as previously stated. In operations for repairs of nerves after loss of substance, attempt should, of course, be made to bring the severed ends in apposition for direct suture, so-called nerve-stretching (Schüller). Even gaps of 4 cm. to 6 cm. are now and then obliterated if slight tension is made on the central and peripheral stumps, especially with the limb placed in slight flexion or extension, depending on the nerve involved. Attention is called to the experimental work of Stoffel, who states that recognition should be given to what he terms "Nervenmechanik," showing how different nerves are affected by limb posture. The median nerve, for instance, is stretched on extension of the forearm, while the ulnar nerve is relaxed. Stoffel has further shown that the funiculi of peripheral nerves have a definite course and position in peripheral nerve trunks, maintained practically through the extent of the nerve. Sensory and motor paths are often quite independent. Attempts should be made in direct suture to bring together the two severed ends in as nearly normal relative positions as possible, so that down growing neuraxes from one funiculus are in favorable position to reach the corresponding funiculus in the peripheral stump. This is said to favor regeneration. It may be added, however, that the necessity of such accurate approximation is not clearly evident as a result of experimental work.

A number of operators recommend surrounding the nerve trunk at the seat of suture by means of fascial, membranous or other tubular sutures. These de-

vices will be considered under the heading of "Tubular Sutures" or "Tubulization" and to avoid reiteration are not considered here.

Various methods have been suggested to bridge the gaps between the severed ends of a peripheral nerve in case there is found too much loss of nerve tissue to bring the ends together and perform direct suture. These methods I shall consider under the following heads:

- A. Nerve flap, or neuroplastic; nerve anastomosis, or grafting; multiple grafting; cross suturing of nerves.
- B. Suture à distance, with catgut.
- C. Tubular suture and tubulization.
- D. Nerve transplantation.

*A. Nerve Flaps.*—Létiévant suggested making a flap from the central or peripheral stump of a divided nerve or from both the central and peripheral stumps, turning the flaps over and suturing them (Fig. 1\*). From purely theoretic considerations, this operation is to be condemned. The central flap degenerates, as do all nerve portions separated from central connections. When

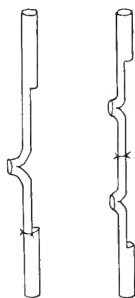


Fig. 1.

regeneration takes place, the central end of the central flap is not in alignment with the central stump and thus offers unfavorable opportunity for the down-growth of the central neuraxes. Should, in spite of this, neuraxes reach the peripheral flap, this again is not in alignment with the peripheral stump. A series of experimental tests of this procedure conducted by me, substantiate these theoretic deductions.

This operation is now and then performed for the repair of loss of nerve substance in man, with variable results so far as reports are concerned. Gratz has recently reported nine cases in which this form of nerve plastic was made. The nerve flaps after suture were surrounded by layers of adipose tissue taken from the region of the wound. Six cases of neurolysis are included in the summary. Eleven cases are reported as giving favorable results. However, the operation is condemned as a result of experimental observations.

In the operation of nerve anastomosis or nerve grafting it is suggested that the central end of the peripheral stump of a divided nerve be sutured against the side or into a normal accompanying nerve (Fig. 2). This operation is, of course, only applicable where two or more large nerve trunks are in close proximity and

\*The figures here presented, give by means of diagram, the operations detailed. In each instance the central stump of the supposed severed nerve is directed toward the top of the page.

have a nearly parallel course. This operation has had some favorable consideration in civil practice, especially among the orthopedists, in cases of paralysis due to central injury of a nerve supplying a group of muscles, largely leg and foot, or again in facial paralysis. The rather extensive literature bearing on this phase of the question is not here considered. On purely theoretic grounds it may be stated that unless the normal nerve is injured at the seat of anastomosis either by denuding epi- and peri-neurium or primarily or secondarily as the result of suture passed through the normal nerve, there is no possibility of a down-growth of neuraxes from the normal nerve into the anastomosed or grafted, degenerated stump. This my own experimental observations substantiate. Manasse has relatively recently reported some experiments in which the peripheral end of the facial nerve was anastomosed on the spinal accessory. On histologic examination, made on tissues removed nearly a year after operation, some medullated nerve fibers were found in the peripheral facial. Clinically and with physiologic tests, evidence of regeneration of the peripheral facial was observed. One can only postulate that in these experiments the spinal accessory was injured at the place of anastomosis, very likely by means of the sutures.

In case such a procedure is deemed necessary, the modification suggested by

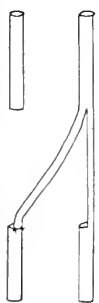


Fig. 2.

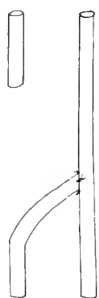


Fig. 3.

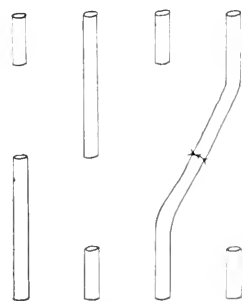


Fig. 4.

Sick-Sänger is to be recommended as presenting at least evident reasons for a possible success. Sick-Sänger suggests, and this was executed in a clinical case, that by means of a sharp knife a flap be made from the normal nerve, leaving the flap attached centralward and suturing its free peripheral end to the central end of the peripheral degenerated nerve stump (Fig. 3). There is every reason to suppose that the outgrowing neuraxes of the centrally attached flap will bring forth neurotization of the degenerated peripheral nerve. It is, of course, to be understood that the normal nerve is injured to the extent of the section necessary to form the flap, and that paralysis in the field of the cut fibers will ensue, probably not wholly relieved by down growth of central fibers. Létiévant has further suggested that in case two approximately parallel nerves are cut at different levels, with loss of nerve substance to the extent that the respective nerve ends can not be sutured directly, for instance, median and ulnar or median and radial, the longer central stump of one nerve be sutured to the longer peripheral stump of the other nerve—cross-suture (Fig. 4). This procedure has been tried experimentally with favorable result so far as concerns one nerve; the other nerve, of course, degenerates. Tillmann has modified this operation by anastomosing

the shorter ends to the longer ends after cross-suture, a modification which on theoretic grounds presents little of value (Fig. 5).

Hofmeister has recently described a method which he terms "doppelte und mehrfache Nervenpiropfung," used in bridging defects where one or several nerves of an extremity or of the brachial plexus are severed with extensive loss of substance (Fig. 6). He speaks enthusiastically of the method, cites 24 cases, in only one of which the results seem at all favorable, the rest not having been observed long enough to admit of determination of ultimate results. The operation consists in making a fine slit in the normal accompanying nerve at the level of the central and peripheral stumps of the severed nerve. The central and peripheral ends of the central and peripheral stumps are then inserted into the slit previously prepared and carefully sutured in place, care being taken to suture only through connective tissue sheaths. If several nerves are cut at the time of injury, the one nearest a normal nerve is double grafted as above indicated, the central and peripheral stumps of other cut nerves may then in like manner be grafted into the central and peripheral stumps of the previously grafted nerve. Numerous diagrammatic figures are given elucidating the several operative pro-

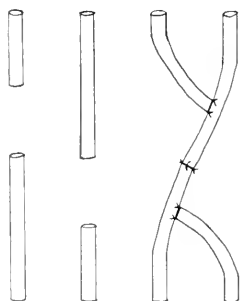


Fig. 5.

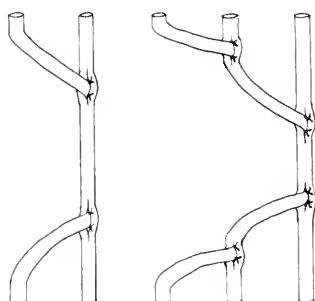


Fig. 6.

tocolled. Hofmeister's contention is not that the normal nerve into which the severed ends of an accompanying nerve are grafted in any way assists directly in the neurotization of the peripheral stump. He suggests, however, that the normal nerve, practically uninjured through this operation, appears a very favored medium for the down growth of central neuraxes and thus favors their approach to the peripheral stump. He further states that flooding of the nerves with a solution of novocaine, 10 cm. of a 0.5 per cent solution, to which 1 drop of suprarenin is added, has favorable influence. It is said to cause temporary swelling and consequent loosening of the constituent tissues of the nerve. The normal nerve trunk is only temporarily affected by this. Bochard gives a case from civil practice in which double nerve grafting was performed.

It is difficult to value this operation in the light of laboratory experience. *A priori*, this operation does not appear to deserve the commendation given it by its originator. It is possible that later reports not accessible to me, giving later details of the operations recorded in the publication cited may give data concerning its value. If this procedure is deemed worthy of experimental test, I should be glad to undertake or direct such experiments. It should be understood, however, that such experiments to be of any value would need, for each operation, to be carried on for at least five to six months.

From what has been stated, it may be observed that the procedures listed under this heading—nerve flaps, nerve anastomosis, nerve grafting, nerve crossing—are, from the viewpoint of the laboratory experimenter, not to be commended; double or multiple nerve grafting as recommended by Hofmeister may deserve a laboratory test.

*B. Suture à Distance (Assaky).—*In this procedure, which was, I believe, first recommended by Assaky, the gap between the ends of a severed nerve are bridged by catgut. Recognition is here given to the fact that regeneration of the peripheral end of a severed nerve depends on the outcome of a struggle between the down-growing neuraxes of the central stump and the developing connective tissue forming between the severed ends. With loss of nerve substance and an appreciable distance between the severed ends, the down-growing neuraxes meet with too much resistance and are diverted from their course before they reach the peripheral end. In experimental work I endeavored to obviate this by placing between the severed ends strands of catgut in the hope that the connective tissue replacing the catgut threads on absorption, would constitute paths of least resistance along which the newly growing neuraxes might descend and thus reach the peripheral stump. Bundles of coarse catgut were tightly entwined with fine catgut threads, the ends of which were kept long enough to serve as suture threads. After thorough sterilization, these bundles of catgut were sutured between the stump of a severed and resected nerve; seven experiments were performed, using the ulnar of dogs. The dogs in three of these experiments were kept long enough to admit of peripheral regeneration; in two of these success was attained. This operation has not been extensively used in surgical practice. The results of the surgical cases reported may be summarized as unfavorable. The operation does not deserve complete condemnation. It is, however, more of academic than practical interest.

Muscle and tendon strips, woolen fibers, etc., have also been used for "suture à distance," with unfavorable results, and need not be further discussed here.

*C. Tubular Suture and Tubulization.*—Tubular sutures, primarily used to preserve a pathway along which down-budding neuraxes may grow, have been widely, though not extensively, used in peripheral nerve surgery. In later times, especially in the recent wars and particularly the present war, tubulization, as often known, has been extensively practiced, not only to bridge gaps after loss of nerve substance, but also to protect the seat of suture and to ensure against interference from secondary cicatricial contraction, especially in what is known as "neurolysis," the freeing of a nerve, not severed in continuity, from the effects of cicatricial contractions at the seat of injury, frequently referred to in literature of recent wars and especially the present war. Numerous substances have been used for the purpose of tubular suture and tubulization, the aim being to obtain a tube which can be sterilized, is not irritant, not absorbed too readily and not subject to secondary contraction.

The following substances may be enumerated as having been used for this purpose: 1, decalcified bone tube (Vanlair); 2, iodoform gauze and epidermis of man (Wölfer); 3, magnesium tubes (Payr); 4, hardened gelatin tubes (Lotheisen); 5, hardened arteries of calf (Foramitti); 6, fascia lata, with or without fat layer; 7, Galabith (Auerbach); 8, Cargile membranes (Sherren).

Consideration of these various devices with their several applications shall now be undertaken.

Tubular sutures by means of bone tubes as recommended by Vanlair, have received considerable attention both in experimental and clinical work. I have used it in eight experiments, ulnar of dog, in four of which the observation extended over a period of sufficient length to admit of regeneration of the peripheral end. The operation consisted of inserting the peripheral ends of a divided and resected nerve into the ends of a decalcified bone tube of suitable size, and suturing the nerve ends in place (Fig. 8). In only one of my experiments was partial success attained. In the other experiments the bone tubes were absorbed too rapidly to perform their anticipated function. Steintal condemns tubular sutures as a surgical procedure as also Auffenberg, Pomeranow and others. The clinical reports and experiments dealing with tubular suture by means of epidermis, magnesium tubes, gelatin tubes, and nonvulcanized rubber drainage tubes, are more of academic than practical interest and their consideration need not occupy space here.

The use of hardened arteries of the calf, for purpose of tubular suture and tubulization has received not a little attention. This procedure was recommended

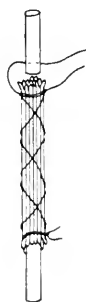


Fig. 7.



Fig. 8.

by Foramitti. He advocates using the carotid arteries of calves, removed at the time of killing, and stretching the same over glass tubes of suitable size. They are then fixed for 48 hours in 5 per cent formalin; washed 24 hours in water; boiled 20 minutes in water and stored as is usual with catgut sutures. Arteries of the calf, treated in this manner, tried experimentally for tubular suture of nerves, proved to be nonirritating, slow of absorption, and do not undergo secondary contraction. This method has been rather extensively used in more recent surgical practice. Hashimoto and Tokuoka report its use in the Russo-Japanese War with favorable results. This method was further used in the First and Second Balkan Wars. However, the reports given are scattered and are not favorable for critical interpretation. Hirschel and also Grosse report on its use in the present war. However, these later reports were made before definite deductions could be drawn relative to its value. This method has had rather wide use in connection with neurolysis. Before closing the wound the freed nerve in the region of the cicatrix is surrounded by a hardened artery of a calf, slit longitudinally and sutured in place,—this with a view of preventing pressure on the nerve as a result of secondary contraction of the cicatricial tissue. This



method by reason of observations made in experimental and clinical work seems to me worthy of consideration. As is pointed out, the hardened vessels can be prepared at leisure and can be stored and kept ready for use in sterile conditions.

Fascial sheaths, more particularly for purpose of tubulization have in recent years received considerable attention both in experimental and clinical work. Kirk and Dean Lewis, two American observers, have recently published extensive experiments on the use of fascia lata for the purpose of tubular suture and tubulization, with favorable results. Pieces of fascia lata of suitable size are placed about the ends of the resected nerve and sutured in place in such manner as to form a tube (Fig. 9). This fascial tube if properly applied does not contract nor collapse. Its lumen early fills with coagulated lymph into which the proliferating sheath cells grow, forming syncytial strands. The results of carefully conducted experiments show that this method deserves consideration, both for tubular sutures and tubulization after neurolysis. However, it must be added that this method is not original with the American experimenters. Denk reports concerning its use in the Balkan Wars; Döffner and Kredel, also, its incidental use by others, in the present war. Details of procedure vary a little with different operators.

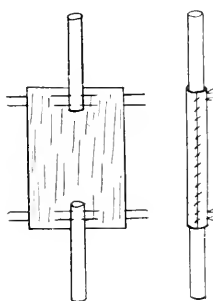


Fig. 9.

Auerbach has recommended the use of Galabith for purposes of tubulization in injury to peripheral nerves. Galabith is a commercially patented preparation, apparently a substance made from casein treated with formalin. Tubes made from Galabith are readily sterilized by boiling for 5 minutes in water (without alkali) which procedure at the same time softens them so that they may readily be cut crosswise or lengthwise. They are said to be nonirritant and are very slowly absorbed. The brief report given contains favorable results. This preparation, only recently recommended, has not been subjected to critical experimental tests.

Sherren some time ago recommended the use of Cargile, fixed peritoneal membrane of the ox, for purpose of tubular suture. I have not seen further mention of its use.

As is obvious from this report, tubular suture and tubulization of injured nerves are methods often combined with other procedures, such as direct nerve suture, neurolysis, plastic operations, and nerve transplantations. Of the methods and materials recommended for use, judging as an experimenter, hardened artery of the calf and tubes made from fascias are to be especially recommended for

clinical use. Fresh arteries and veins, used as auto- homo- and hetero-transplants, for purpose of tubular sutures and tubulization, here and there recommended, offer no advantage over fixed vessels, and present certain disadvantages, owing to the fact that they readily collapse, therefore, need no further mention here.

Attention may yet be called to the use of celloidin tubes in the experimental work of Forssman, with results, so far as concerns tubular sutures, of academic interest only.

*D. Nerve Transplantation.*—The use of segments of nerves to bridge defects after nerve injury with loss of substance in peripheral nerves, is familiar to the experimenter and the clinician, and has received extended attention by both. The method was suggested by Philippeaux and Vulpian and tried experimentally, then used by Assaky in clinical cases, and by Huber in a long series of experiments. Of 26 experiments made on the foreleg nerves of dogs, 10 were observed long enough to admit of regeneration of the peripheral stump, of which 5 presented complete, and 4 nearly complete, return of function. Of all the methods used in my experimental study of bridging defects in peripheral nerves, those of nerve transplantation gave the most favorable results. It has not been possible to review *in extenso* the large surgical literature having reference to nerve transplantation, owing to my lack of familiarity with this literature. This was done by R. Peterson (1899), Sherren (1906), Kilvington (1908). The results of clinical cases seen in reports are on the whole favorable, the more favorable the longer after the nerve transplantation the report was made. The reports, having reference to recent wars and to the present war, contain few references to cases of nerve transplantation, so far as the literature is accessible to me.

Recent experimenters have drawn attention to the necessity of distinguishing in results obtained in nerve transplantation between such as constitute auto- and homo-transplants and heterotransplants. Sherren reports 50 per cent successful out of 8 cases of auto- and homo-transplants and 37 per cent successful out of 22 cases of heterotransplants. Kilvington speaks of more favorable results in homotransplants. Forssman and again Merzbacher were the first to suggest the important differences between homo- and hetero-transplants. Verga, as also Maccabruni, believes the results to be the same whether homo- or heterotransplants, are used. Ingerbigsten, who reports recently on experiments with reference to this question, speaks in favor of using homotransplants. The question of using auto- or homo-transplants of nerve segments in preference to heterotransplants, in clinical cases, on the basis of experimental and clinical observations can be decided in favor of auto- or homo-transplants in case suitable fresh nerves from man are available at the time of the projected operation, and it should be borne in mind, that especially in secondary operations for loss of nerve substance, both the time of the operation and the method used are often a matter of choice. It should be stated, however, that heterotransplants, transplantation of nerve segments taken from an animal of another species than the one to which the transplant is made, is an operation not to be wholly condemned, since clinical observations and experimental work both justify this operation. In conclusion it may be stated that both clinical and experimental observations

warrant the deduction that the operation of choice in cases of repair of an injured peripheral nerve, where direct suture can not be obtained, is the operation of nerve transplantation, especially if auto- or homo-transplants are available. This may be combined with tubulization by means of fascial sheaths or hardened arteries. Of the methods of tubular suture and tubulization, hardened arteries and tubes of fascia are to be given preference. Experimental observations with other methods do not warrant their recommendation. Concerning the use of Galabith I know of no experimental observations. The same may be stated concerning the method of double or multiple grafting suggested by Hofmeister.

The literature cited, only the more pertinent of that reviewed, contains citations, especially with reference to nerve suture as observed in the present war, many of which deserve study by clinicians, more particularly neurologists, with reference to diagnosis and after-treatment, for which my own work has not given the necessary training, to present a critical review.

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## WAR DEAFNESS AND ITS PREVENTION—A CRITICAL REVIEW\*

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WAR deafness is of importance from at least four standpoints: (1) trained men are incapacitated for service for shorter or longer periods and must frequently be discharged; (2) the handling of them takes the time of doctors, nurses, and equipment which would otherwise be available for other work; (3) the victims are handicapped in later social and industrial life; and (4) they become eligible for pensions of larger size. These are all economic reasons, and do not take into account at all the policy of the State to do all in its power to protect its soldiers from unnecessary hardships. The consideration of war deafness and its prevention is therefore of interest to the nation and to the world at this time.

In previous wars the cases of deafness have for the most part been confined to the artillery and the navy, where the proximity of large caliber guns when fired gives rise to great air disturbances. Until the present conflict, the ammunition, other than small-arm projectiles, most used against troops has been shrapnel, and this normally explodes at a distance too great to inflict damage to the ear by air pressure changes. Also the bursting charge of shrapnel is small. But in the present war the increased use of high explosive shells and hand grenades, which explode on or in the ground, has put infantry in a position where the excessive noises and air pressure changes are greater than with the men serving large guns, except when they are also under hostile fire, or when they are too near the muzzle of a gun. According to Friedländer, in the Franco-Prussian War there were only 12 cases of indirect injury to the hearing in the Prussian army; in the Russo-Japanese War only 101 cases were reported on the Japanese side. Friedländer's figures must be for the Japanese army only, since Suzuki† reported 116 cases in the navy alone. In the present war no statistics are yet available, but a review of the literature in which cases are reported by English, French, Italian and German physicians, indicates that the cases are to be finally counted in tens and even hundreds of thousands, since many of these men have seen hundreds and even thousands during the first year of the war or during some shorter period which their report covers. These reports deal fully with differential diagnosis of the various types of disturbance, the treatment of each, and the prognosis; but in most cases nothing at all is said about preventive measures, and when it is mentioned it is usually only a paragraph or less of a very general nature. It would add to the value of these clinical reports if they included a statement as to the use or nonuse of prophylactic measures in injured cases and as to the measure used. It is the purpose of this paper to deal primarily with prevention, so these clinical papers will not be referred to in detail. It suffices to say that they are numerous already and new ones appear every month. This, together with the fact that so many devices

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†Quoted from Horne.

have been suggested and are at present in use, and the further fact that most men seem to use nothing, simply means that no satisfactory preventive measure is recognized and made available. An editorial writer in the *British Medical Journal* of March 24, 1917, states that "at a recent meeting in Germany, at which concussion wounds of the tympanum were discussed, it was authoritatively stated that no satisfactory device had yet been found to prevent this and allied injuries." The authority for the statement is not given; if correct, the Germans are also still searching for a prophylactic measure.

Indirect injuries to the ear in war are divided into two groups: (1) those that are due to the continual noise and are gradual in development; and (2) those that are caused by a single detonation. "Noise deafness" or "gun deafness" of gradual development is undoubtedly the same condition as "professional deafness" of boiler-makers and others working in noisy trades, and the preventing of these cumulative injuries by the use of any device that permits conversational sounds to pass seems improbable, though probably the degenerative changes of the cochlear parts would be rendered more gradual. Complete cutting out of the sound waves is out of the question with troops, for it is necessary to have the hearing of orders as little interfered with as possible. But the sudden injuries due to high air pressure, rather than to sound waves, are theoretically preventable; and it is this group of injuries and means for preventing their occurrence that will be especially considered.

Detonation effects on the ear may be divided into three groups: (1) rupture of the tympanic membrane, (2) organic disturbances of the labyrinth, and (3) functional disorders, probably of the central nervous system. The numerous clinical reports agree that each of these may occur alone or in combination with one or both of the others.

The rupture of the tympanic membrane is due directly to the difference in air pressure on the two surfaces of the membrane; since the eustachian tube is not normally open except during the swallowing act, the air in the middle ear remains at normal pressure while that in the external meatus may be increased to twice normal or more by an explosion. Wilson makes the statement, on what authority he does not say, that the air pressure in the vicinity of exploding shells is frequently raised 10,000 kilos per square meter. This, when reduced to units we are accustomed to use in considering gas pressures, is equal to 73.5 cm. of mercury; and is, in round figures, equivalent to doubling the pressure on one surface of the membrane. That this much pressure is sufficient to cause the rupture of even normal membranes is evident from the experiments of Zalewski, who has shown that there is a considerable range of variation in apparently normal membranes. He secured 232 temporal bones at autopsy; of these, 112 showed apparently normal membranes. With air pressure gradually raised he found the average bursting pressure to be 120.9 cm. of mercury, but some (10.8 per cent) burst at less than one atmosphere (76 cm. Hg.). Twelve membranes with thin spots ruptured at an average pressure of 42.83 cm. Hg., and twelve membranes with scars, at 22.08 cm. Hg. In 20 cases of acute infection of the middle ear the average pressure required to burst the tympanic membrane was 78.55 cm. Hg. Even if no allowance is made for the possible difference in bursting pressure required when suddenly applied by detonation as compared to

the gradual increase of pressure used in the experiments, it is seen that in an average group of men there are many with membranes that will burst at pressures produced in the neighborhood of a bursting shell. It is certainly not feasible to eliminate from the army all those with tympanic membrane defects, and even if this were done, it would not stop all of the rupturing of tympanic membranes. The perforations are almost invariably in the pars tensa and usually in the lower part. With modern technic the treatment required to secure a healed membrane with perfect restoration of function is readily given, and the prognosis is good if infection of the middle ear can be avoided. Unfortunately in the dirt and filth of combat life, this infection frequently occurs, with the resultant injuries; and in all cases, infected or not, the individual is of necessity incapacitated for duty for a considerable period of time, and requires services of the medical corps, usually already overworked.

The organic injuries of the labyrinth by detonations are fairly well known since the work of Wittmaack (1907) and those who have modified his methods. While in these experiments with animals Wittmaack, Yoshii, and Hoessli all used small firearms, the effects on the ear are doubtless similar to those that would be produced by large guns and by shells, for they compensated by working at short distances. Recently Prenant and Castex (1916) placed animals in cages near cannons at Fountainebleau. That these animals were subjected to conditions of the type which cause gradual changes rather than the type causing detonation effects is evident from the fact that in no case was the tympanic membrane ruptured or the mucous membrane of the middle ear inflamed. They found extensive cochlear lesions of the type to be expected from previous work in this line, though they make no reference to other work and apparently consider the experimental causation of such injuries a new thing. Yoshii (1909) has made the most extensive study of the effects of detonations on the inner ear, using a revolver fired near the animals. He used two series of guinea pigs. With one series only one detonation was given, after which animals were killed at once and after 2, 3, 8, 25, 45, and 60 days. The other series were exposed to detonations daily and animals were killed after 3, 5, 15, 20, 25, and 30 days. It is the once exposed animals that are of most interest here; and for presenting the nature of the injuries to the inner ear caused by detonations rather extensive quotations from Yoshii's\* reports will be used. The injuries are doubtless similar in man.

"Pathologic changes occur simultaneously in all parts of the organ of Corti. The outer and inner hair cells are greatly swollen, they have lost their characteristic form and structure and are in part loosened from their support. The nuclei are pushed upwards and frequently are broken into small fragments. Also the Deiters' cells lose their structure and become changed into a homogeneous mass. Their supporting fibrils perish. The pillars remain but become bent and appear only indistinctly. The narrowed tunnel space is filled with a homogeneous mass, which stains faintly with eosin, hence appears like extravasated cell contents. Nuel's space and the outer tunnel space are no longer present; the cells of Hensen are deformed, flattened out. Frequently a great space is found between the loosened hair cells and the curve of the cells of Hensen.

\*Translation, page 228 ff.

"These changes are all found immediately after the first shot and reach their height after two to three days. Then a regeneration process sets in slowly. Thus we find the organ of Corti already somewhat improved in the animals killed 8 days after the shooting. First the bent pillars straighten out and are recognized clearly. The hyaline mass in the tunnel is resorbed, and the space itself restored. The hair cells, Deiters' cells and their supporting fibers are not clearly differentiated yet in this stage of repair. The healing process does not proceed in all turns evenly, while the basal turn already shows a bettered condition, in the other turns are found yet evident disturbances.

"In after days the regeneration process proceeds further, the cells of the sulcus spiralis externus and internus and the cells of Hensen take their previous form. The restoration of the hair cells and the Deiters' cells seems to be accomplished only very slowly and incompletely; for the animals killed 60 days after the shooting showed only in the basal turn the hair cells and Deiters' cells in an improved, but nevertheless not fully normal condition, while in the remaining turns clearly differentiated hair cells and Deiters' cells can not yet be demonstrated."

Yoshii further states that with repeated shooting day after day the degeneration proceeded until Corti's organ was replaced by a flat epithelium, as in any noise deafness and in animals exposed for a long time to a single note. This severe change was limited to the region of the transition of the basal to the second turn. The tectorial membrane also was changed in position and structure. The stria vascularis showed minor changes. In cases killed at once after the single shot, blood corpuscles and free hemoglobin were found in the perilymphatic spaces. This was resorbed in time. The epithelium of the maculae and cristae acusticae showed a slight swelling. The ganglion cells of the spiral ganglion and the nerve fibers of the same undergo very severe alterations, but in the single detonation cases the ganglion cells had at the end of 60 days returned to a nearly normal condition. In the repeated shooting cases, as in noise deafness, they completely disintegrate.

From the above description of the changes that occur it is very easy to understand the typical clinical picture, with gradual improvement to certain sounds and even to all but with permanent hearing defects in many cases. From the nature of the lesion produced, it is evident that the only remedy is prevention, for we can not cure it.

As to the nature of the changes in the "functional deafness" cases, very little is known. The hypothesis advanced by Wilson (1917) is interesting, but he claims no proof for it. So here again prevention is the best remedy.

As stated before, all three of these types of injuries may occur together, or any two together, or only one. The organic lesions of the cochlea are the most common, and the general impression of the clinicians is that the labyrinth injury is less in those cases in which the tympanic membrane ruptures than in those in which it remains intact and so transmits the full force of the concussion to the labyrinth.

The factor causing the tympanic membrane rupture is certainly the increased air pressure; and the most probable cause of the other two types of injury is the same, but a few workers have maintained that bone conduction of vibra-



tions from the ground or other support of the body to the internal ear is necessary to cause labyrinth injury. For this reason it is necessary to sift this evidence, for preventive measures to be effective must be suited to the etiologic factor.

The experiments of Wittmaack (1907) on guinea pigs furnish the chief support for the bone conduction theory. This worker at first employed a continuously sounding electric bell placed in the cage near the animals, and killed them at intervals of from 5 to 60 days. The results were negative. He then connected the bell in such a way that the clapper caused the sheet iron base of the cage to vibrate, as well as producing air vibrations, and thus submitted the animals to a combined air and bone conduction. The cochleæ of the animals of this series showed very marked injuries to the nervous elements and to the organ of Corti. The animals were so affected by the continuous vibration of the cage that the experiment was forced to end after 16 days. Accordingly experiments were made in which the sound was maintained from 10 to 14 hours daily, and in these series the general health of the animals remained good and they even gained in weight. Characteristic degenerative changes were found in the spiral ganglion cells, the nerve fibers and the organ of Corti. Wittmaack concludes from these experiments that bone conduction is essential to the injury of the labyrinth by sound waves. In 1909 von Eicken reported experiments on guinea pigs with the incus removed on one side by operation, so that one side retained normal conduction and the other mainly bone conduction. Part of these animals were submitted to the action of a *c* organ pipe, others to a *g* pipe. The cochleæ of the *c* animals were negative on both sides; those of the *g* animals showed degenerative changes on the normal side in a circumscribed area, while the operated side remained normal. In 1911 von Eicken presented further evidence of the same nature. Hoessli (1912) combined the use of guinea pigs with the incus removed on one side with the use of a specially constructed apparatus designed to duplicate as nearly as possible the working conditions of boiler-makers. The animals stood on the inside of a section of large iron pipe which was struck by hammers operated by machinery. Thus both air and bone conduction factors were present. Hoessli found that injuries to the cochlea are constant where normal middle ear parts are present and just as constantly the side with the broken middle ear chain remains normal. As controls, animals with both ears normal and ones with the incus removed on both sides were employed. In 1913 Hoessli reported further work with similarly operated animals which had been placed on a felt pad in the tube as a test of the prophylactic measures recommended by the Wittmaack group of workers. He found that the results were the same as when the animals stood directly on the vibrating pipe; ears with normal middle ear apparatus received injuries to the cochlear parts, those with the incus removed were not injured. He thus advanced strong arguments against bone conduction being essential to injury. Previous to the work of Hoessli, Friedrich (1907) reported on the testing of the hearing of 19 naval officers; he found an average injury greater than Müller (1899) did with foot artillery officers, and on the basis of Wittmaack's newly reported experiments he concludes that the vibration of the frame of the ship is responsible for the greater injury in the case of the naval men, and recommends some form of padding to deaden the conduction of the vibrations of the ship to the bodies of

the men. Jaehne (1911) investigated clinically the hearing ability of 61 artillery officers of several years' service, and found injuries to about 70 per cent of them. On the basis of Wittmaack's experiments and of the positions of the men with various duties and the average injury to each group he concludes that bone conduction, rather than air conduction, is the injurious factor. Friedländer (1915), on the basis of Hoessli's experiments and his own clinical experience, concludes that air conduction is the injurious route. Bogdanoff-Berezovski\* (1914) criticizes Hoessli for transferring the results of his continuous sound experiments on animals with the incus removed to detonations on the normal ear, and argues that bone conduction may be very different than with less intense sounds, but he brings forth no proof for his opinion. However, considering the whole question of prevention open, he says he asked a physician in the navy, A. A. Miasoédov, to undertake experiments with troops on ships, and he says the work is under the immediate supervision of N. P. Simonovski and that the greater part is finished and there is left only to wait for the conclusions on working out regular preventive means.† Siebenmann (1915), in whose laboratory at Basel Yoshii and Hoessli worked, in a review of the experimental work on sound injuries of the ear, concludes that the use of felt pads, padded shoes and rubber gloves for protection on the basis of bone conduction, is of no avail.

Hoessli's work at least proves that bone conduction alone is not injurious as soon as the normal arrangement is. He also tried plugging the external meatus of guinea pigs to exclude air conduction that way; but all of his cases developed an infection of the middle ear, which renders the results of little value, though they were negative in all cases that were plugged. The plugs were worn continuously and fastened in with glue to keep the animals from scratching them out; this continual use was probably a factor in the infections. Other plugging experiments of Hoessli will be referred to later. Attention should be called to the fact that the experimental work mentioned has really been on injurious sounds which cause gradual, not sudden, changes. In all of the experimental work in which detonations have been used, normal middle ear parts have been present, and no evidence has been found that indicates that bone conduction plays an important part in producing detonation deafness, though the general body shaking it in itself unpleasant. The conclusion must be that the evidence at hand clearly indicates that the search for a prophylactic measure to prevent detonation deafness must be directed toward the prevention of injurious air conduction.

The more severe systemic reactions known as "shell shock" are also due to the high air pressure in the vicinity of an exploding shell and in the most severe form the result is instantaneous death, with no external lesion at all. Examination of the tissues from these cases has shown the existence of microscopic hemorrhages, especially in the central nervous system and the kidneys; and in the nonfatal cases bloody urine is a frequently recorded observation, together with a high albumin content of the cerebrospinal fluid. Recent work also indicates a secondary suppression of the adrenal secretion as responsible for some

\*I desire to thank here Prof. C. L. Meader of this University for his kindness in translating this Russian article.

†I have looked in vain for any mention of this work mentioned as being nearly ready for publication in March, 1914.

of the later symptoms. These more severe effects of detonations are mentioned here in this general way in order to indicate how they differ from the deafness effects, and to point out that from the very nature of the lesions the prophylactic measures for the ear parts can not be expected to have any beneficial action in preventing these other injuries.

Opening the mouth during the discharge has been a common practice for a long time with men handling large guns. It is based on the idea of permitting the pressure on both sides of the tympanic membrane to remain equal, but at best it is only partially successful, because of the relative size of the external meatus and the eustachian tube and the fact that the pressure changes are sudden, not gradual as in caisson work. In many cases the eustachian tube is not patent even during swallowing, owing to chronic or acute inflammation of the nasopharynx and tube. Many men chew something in order to aid in opening the tube when wanted, but both in practice and in theory this method is lacking; and for troops under fire it is entirely useless, since the time of a dangerously near shell explosion can not be anticipated and prepared for as the gunner can prepare for the discharge of a gun.

Placing the finger tips in the ears is also a common practice; but for the above reason of unexpectedness of danger is of little avail; also, soldiers in combat have other uses for their fingers. It has further the danger of infection of the ears from dirty fingers, but it is a practice which will be frequently used as long as no other protection is provided. Many writers have discussed both of the above mentioned measures; they are not referred to separately; all agree in the futility of the procedures.

The use of cotton to plug the meatus is the most common procedure of all. It has the advantage of being cheap, usually available, is easily placed and the degree of occlusion may be varied by the firmness of the packing. The consensus of opinion among medical men for some years has been that dry, air-containing cotton is of little use; when wet so as to fill the air spaces it is of value if placed firmly, and it is used with various oils as a means of filling the spaces. No statements have been found on observations from any source as to the relative effectiveness of the various ways of soaking the cotton used; this, doubtless, has a large personal element in it, depending on the firmness of the packing and the thoroughness of the removal of the air by careful soaking and placing of the plug. Hoessli (1912), in the course of other experiments, stopped the ears of two guinea pigs with wet cotton and fired a heavy revolver five times in rapid succession about 30 cm. above the animals. In one animal no changes occurred in the inner or middle ear of either side, in the other animal the left ear showed traumatic effects which Hoessli thinks were probably due to the animal having succeeded in partially scratching out the cotton, so that the occlusion was not perfect. In control animals without protection there was a crushing of the organ of Corti in the lower part of the second turn of the cochlea, with the outer hair cells and Deiters' cells entirely destroyed. The animals were killed 24 hours after the exposure. Hoessli then used four cats in a similar experiment, stopping the left ear of each with wet cotton, and to prevent the scratching at the plugs he narcotized the animals, then shot the revolver five times at a distance of 25 cm. from the heads of the animals. He killed them after 24 hours and found the left ears normal in all cases, while the right ears

in three cases showed positive, though slight, changes; the fourth case had a normal right ear. Hoessli considers that for cotton to be effective it must have the air spaces filled and must be tightly packed, and he considers the lack of protection afforded in the army use of it, based on the reports of Müller, Friedrich, and Jaehne, especially, to be due to the use of lightly packed air-containing cotton. Friedrich's report has already been given. Müller (1899) examined the ears of 51 men before and after shooting practice with 9, 12, and 15 cm. guns, from 50 to 90 shots being fired. Müller says that he wanted to have a comparison of the effects with and without cotton, but could not because of the strict enforcement of the German army regulations requiring artillery men to use cotton plugs during the firing of guns. His examinations were made immediately after the shooting, so, doubtless, many of the effects observed were only temporary; but he states that while all the men used cotton and stayed back from the guns a distance of 3 to 5 meters during the discharges, 44 of the 96 ears examined showed injuries of greater or less degree, though none severe enough to incapacitate for work. He says that the post physician, Grässner, credits the rarity of tympanic membrane ruptures among the artillerymen in recent years (preceding 1899) to the enforced use of cotton; he had only one case the year before. Jaehne's (1911) report on the injuries to the hearing in a group of 61 artillery officers, these being all the officers of a regiment, irrespective of whether they thought they had been affected or not, includes a comparison of the use and nonuse of cotton plugs, and is alone in that respect among clinical papers. He states that he does not find the regulation obeyed; of the 61 officers, only 24 claimed to have used cotton regularly, 32 had used it irregularly, and 5 said that they never used it. Of the 24 who used cotton regularly, 20 had injured hearing and 4 were normal. Of the 37 who used cotton irregularly or not at all, 22 showed injuries and 15 had normal hearing. Jaehne thinks that cotton does afford a degree of protection to detonations, but does not influence the course of labyrinth injuries of the gradual type. He thinks that this is probably due to bone conduction, for the reasons already given. As previously pointed out, he really was dealing with the effects of repeated sounds, not heavy detonations; and in his summary, while stressing the use of some nonconducting material between the body and the source of sound, he recommends retaining the use of cotton because of the danger of being near the muzzle accidentally. Friedländer, (1915), in a long clinical report and discussion, favors the use of cotton with oil, glycerin, or vaseline; but says it renders one very hard of hearing. Lannois and Chavanne (1915), in a single sentence in a clinical article, say that they consider cotton to be the only practical measure in campaign, but admit that it is not perfect. Bogdanoff-Berezovski (1914) in his conclusions says that while awaiting the report of Miasoëdov the use of both vaselined cotton in the ears and of pads of nonconducting material on the shoes is to be recommended. Siebenmann (1915) thinks that the real protection is afforded by the plugging of the ear with an air-free mass, such as oil-saturated cotton or some form of antiphon. He specifies no particular kind of antiphon, and, as this word is generally used loosely to refer to any device for deadening sound, his meaning is not clear except as to the cotton. Wicart (1916) recommends stopping the ear with cotton soaked with glycerin and then covering the whole mastoid region and the external ear with cotton. He states that all his cases of

more than temporary injury had previous ear trouble, but the majority of clinical reports do not support this view. Based on this idea he further recommends that until the military examination weeds out at the recruiting office all with any ear trouble history, glycerin be put in the external meatus daily and cotton only at the time of combat, the glycerin serving to keep the cerumen soft and to minimize the inflammations that develop. He also recommends daily throat disinfection. There are many more references to the use of cotton, but they bring out no points not already made, so will not be mentioned separately. Cotton mixed with plasticine as used in the British Navy comes under the head of plastic obturators and will be considered there.

Solid obturators of many shapes and materials have been made. Some are placed in the external meatus, others are held against the orifice by special devices fitting in the folds of the pinna. Luzzati (1916) raised two objections to all solid obturators: (1) the hardness of the material irritates the skin and causes inflammations and even eczema, and (2) they project and so may become the source of severe injury to the wearer when he is thrown or falls against a wall or on the ground, and the playfulness of companions who poke them also is a source of injury. To these objections of Luzzati I would add a third; in combat bullets passing through the outer end of the meatus or grazing it and wounding only the pinna, causing only slight wounds in themselves, would cause much more severe wounds if a solid obturator were present to fracture and form secondary projectiles. This is not an unexistent danger, for direct wounds of the external ear are frequent in this trench warfare. Solid obturators will not be taken up further because the objections to them are too great in comparison with other measures.

Of the various mechanical devices which have been patented, only a few are suitable for military purposes. The Elliott "Ear Drum Protector," in both the original form patented in 1904 and in the modified form patented in 1912, has been purchased and used by many naval and artillery officers and men, both in this country and in foreign countries. In the type now manufactured the essential principle is that of a double diaphragm with a tube in the center passing through and supporting both diaphragms, with a narrow air passage in the tube that has openings between the spaced diaphragms and at the inner end, designed to permit slow changes of the air pressure, but not large enough to permit of raising the air pressure rapidly enough to be dangerous during the brief time a detonation lasts. While, of course, it interferes with hearing, it transmits orders and conversational sounds sufficiently well. Considering the length of time it has been used, it is odd that so little is to be found concerning its efficiency. The only report of it in use that was found is that of Dunbar (1913), who, in an article on naval sanitation in general, devotes a paragraph to gun deafness and says in part: "Several appliances designed to break the force of the concussion on the tympanic membrane have been tried, and while the Elliott ear protector is not entirely satisfactory, it has proved the best so far tried." The Mallock-Armstrong "Ear Defender" is a mechanical device invented by Mallock for his personal use about 1895. The U. S. Patent Office Gazette records that it was patented in this country Aug. 3, 1915 (application filed Feb. 12, 1915) so it appears that until this war it was not exploited or used much. A description was published in the *Lancet* of Dec. 26, 1914, and in the *British Medical*

*Journal* of Jan. 2, 1915. It consists of an ebonite cylinder made in four sizes with a larger outer end in which is fitted a diaphragm of thin metal and on either side of this is heavy metal gauze, these gauzes being spaced from the diaphragm a short distance. The diaphragm movements for ordinary sounds are small and so it hinders them only slightly, but large excursions of the diaphragm due to great air pressure changes are prevented by the gauze backings. The theory is good, the drawbacks are those already mentioned as common to all hard obturators, given in the preceding paragraph. The first objection can be overcome, I believe, by using smaller tubes and covering them with a wax layer that is moldable at ordinary temperatures, so that the meatus can be closed effectually without having the plug fit so tightly as to be irritating. Another objection common to both of the mechanical devices mentioned is the price; the Elliott protectors retail for one dollar a pair, the Mallock instrument for three shillings, in London. Friedländer's only reference to the Elliott device is that its cost prohibits its use in the ranks. Doubtless both types would sell for much less if produced in quantities sufficient for a whole army. From the structure of the Mallock defender I think they would not last very long in field service; dirt would clog up the gauze and this would get bent and come in contact with the diaphragm and would soon fail to transmit ordinary sounds clearly, and so would not be used by the soldier. A continual supply of new ones would be required to replace old ones; this would be an important cost item. From their structure the Elliott instruments would seem less liable to get out of order, and if the small canal did get stopped with dirt it would be cleaned and used again. Only one reference to the Mallock instrument in use has been found; that is in the form of a letter to the *British Medical Journal* by Abercrombie, who quotes from a letter received from a relative who was an artillery officer. The quotation is a very enthusiastic endorsement after personal use; apparently several officers in his command had them. The Frank "Ear Stopple" is mentioned by Dunbar as having been recommended for use in diving and swimming, which is its intended use, and also for reducing concussion due to gun fire. The devices were patented in 1908 and consist of a soft rubber tube with an outer flange and closed at the inner end by a thin diaphragm of rubber. The diaphragm is not heavy enough to resist the air pressure changes of concussion sufficiently to protect the ear, and as at present made it would seem impossible to modify them so as to suit the need. Eysell\* has described a small plug containing "a cavity guarded by a metal valve, which transmits ordinary sounds but closes automatically when the atmospheric pressure is raised by explosions." No report is made of its effectiveness in actual use. There have been several complicated plug arrangements described and patented, but the ones mentioned are the only ones at all suited to military purposes, though the others may be useful in complete exclusion of sounds.

Plastic obturators have been made of various wax mixtures, and these are, in my opinion, the most satisfactory of all the devices. A plug made of a material that is soft enough at ordinary temperatures to conform exactly to the shape of the meatus closes the passage perfectly without fitting so tight as to

\*This article appeared in the *Münchener medizinische Wochenschrift*, which has not been received at the library here for over a year. The statement made is based on an editorial in the *British Medical Journal* of March 24, 1917.

irritate, and if the consistency of the mixture be right it is yet rigid enough to transmit the sound vibrations and at the same time firm enough to resist successfully the great pressure changes of the air during detonations. And it has the further advantage of being possible to render it antiseptic by the addition of suitable elements to the mixture. Furthermore, a bullet passing through the region of the plug will not shatter it and form more projectiles of the protective instrument itself, as with solid obturators, but will penetrate evenly as in soft tissues of the body, and if the plug has been impregnated with an antiseptic the pieces carried into the wound are not in themselves a source of infection. The British navy has for several years recommended a mixture of plasticine with cotton, and the *Lancet* of May 6, 1911, reports the First Lord of the Admiralty, Mr. McKenna, as saying, "Tests have been carried out on various preventives, but opinions differ very widely as to their relative efficiency. An ear paste is already prepared and is issued when demanded." No reports on its efficiency have been found, but it can not have been entirely satisfactory, or the British would not still be trying other things. In the *Lancet* of Aug. 15, 1914, two weeks after the war began, Horne stated the requirements of an efficient ear plug, and said that what had been used for some time was of the consistency of jeweler's wax and not antiseptic. He probably refers to the paste above mentioned. In a letter in the *Lancet* of the following week, Aug. 22, 1914, Horne states that a Mr. F. Rogers, of 327 Oxford Street, London, W., has produced a plug meeting the requirements that he set up in his article, but he does not describe it. An article in the *British Medical Journal* of Jan. 23, 1915, states that samples received from Rogers were "composed of plastic materials held together by some fiber, and the mass impregnated with antiseptics." The statement is also made that "conversation is not much interfered with"; and that they do not prevent conversation on the telephone, an essential point for the military officer. In an editorial in the *British Medical Journal* of Jan. 2, 1915, it was stated that "so far as we have been able to test them, and the tests have been conducted not only on civilians, but also upon soldiers from the trenches, the plastic ear plugs afford more protection than the Mallock-Armstrong Ear Defender against very loud and sudden sounds." The price is given as one shilling per pair, postage extra; this is also an advantage in their favor. The Italian Navy, according to Luzzati (1916), has adopted a plastic obturator, after testing out with men on a ship during target practice in 1915. Following the suggestion of Rho (July, 1915) that a wax mixture would be best, Gianturco (August, 1915), a pharmacist, made mixtures of wax, paraffin, almond oil, turpentine, and other things, in varied proportions. He obtained mixtures of wax, paraffin and almond oil that soften at suitable temperatures for both winter and summer work.\* The mixture, finally made use of, because of the scarcity and cost of the low melting point paraffin needed for Gianturco's best mixtures, is given in a note by Rho as being yellow wax with 36.4 per cent of liquid vaseline. This mixture is placed in cone-shaped gauze bags, and Rho said that the work was to be done at the Pharmacy of the Naval Hospital in Spezia by women of the civil mobilization. There is no mention made of antiseptics being added.

\*A comment of Gianturco on mixtures containing turpentine is worth repeating for the benefit of any who may try these things; he found it too volatile; it evaporates and lets the wax harden in lumps; and he also was not successful in overcoming the irritation it produced on the skin of the meatus.

Luzzati says that the officers and men who used them during the target practice found that they gave adequate protection against the painful effects of firing, and did not interfere greatly with hearing orders. Gianturco gave the cost of the mixture he recommended at 4 lire per kilo, and as Rho substituted a cheaper mixture, the biggest item here is the handling in filling the gauze sacks. The number that can be made from a kilo of material is not given, but these are certainly the cheapest of all the specially prepared plugs. Friedmann (1909) describes a plug made by Küppers, consisting of a sphere of wax modeled around a twisted silver wire with a projecting loop large enough to prevent it from getting in too deep, and useful also in grasping to remove the plug. Halle (1913) described an "antiphon" made by dipping a piece of cotton in melted paraffin and letting it cool, the string to be used in removing the plug. The conical plugs of Rogers and of Rho have the large end out enough to be grasped by the finger tips for withdrawal. The gauze container of the Italian type is a valuable idea, as it overcomes one of the objections raised to the others; namely, the danger from broken-off pieces remaining in the canal and acting as an irritant.

In the preparation of this review access has not been had to the military records of this or other countries, and it is very possible that these records contain reports of tests which indicate definitely some prophylactic as being the best. If so, it is to be hoped that the proper officials will utilize such knowledge while it can be of the most service.

I realize fully how unsatisfactory the conclusions to be drawn from this review are; the need of more work is evident. Especially needed are reports by those handling wounded at the front as to the kind of preventive used by those injured and the injury received while using; and these reports will be more valuable if the percentage of injured of all those using each different type can be determined. But while gathering and digesting the papers here presented and the clinical papers not given, a few convictions have been formed which I wish to add to this review. In the first place, the issuance and compulsory use of some preventive, even if it be nothing more than cotton with vaseline, is in the interests of efficiency in the army; and, as stated in the first part of this communication, of economic importance. In this war of attrition, even such small things as ear plugs are of importance if they can serve to keep trained men in active service. Shall we refuse to use what is at hand because it is not the perfect preventive yet to be found? Certainly we should not continue to leave to the individual soldier, uninformed in this matter, the providing of preventives for himself. It is fair neither to the soldier nor to the nation. Nor should such preventives as we have be issued only "when demanded" and to those few who realize the danger and know of the preventive, but they should be issued like clothing, rations, ammunition and other equipment; to all the troops, officers and men alike, and their use not left optional but made compulsory when going into action. Like any other preventive, the object of their use is to keep the active service list up and the hospital list down, and the use of most preventive measures is not left optional in military life. All are efficiency measures; and I believe that the increased efficiency of an army using plugs would be many times greater than the cost of the plugs; they would be one of the smallest expense items of all the equipment of a soldier, and the bulk of even large numbers would not be a very noticeable factor in the problem of transporting supplies.



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## THE FOURTH VENEREAL DISEASE—EROSIVE AND GANGRENOUS BALANITIS\*

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IN the course of routine examinations of genital lesions for the *Treponema pallidum*, we have recently encountered six cases showing a bacteriologic picture of the condition described in this country by B. C. Corbus,<sup>1, 2</sup> of Chicago, under the name of erosive and gangrenous balanitis.

An examination of American medical literature and textbooks fails to show any description of this disease, a condition difficult to understand, as Corbus called attention to this very typical type of infection as early as 1909, and since the infection had been recognized as a separate clinical entity by foreign observers for some years preceding the publication of Corbus's first article.

Corbus considers the condition to be relatively uncommon among private patients, but to show an incidence of about .5 per cent among dispensary patients affected with venereal sores. He has informed me<sup>3</sup> that he finds the infection to be a very common affair among patients in the Central Free Dispensary of Rush Medical College, and that a number of his colleagues have reached the same conclusion. In general, however, the disease as a distinct clinical entity, is but little appreciated among the members of the medical profession.

We have found six cases among about five hundred patients with venereal sores, and as the gangrenous type may lead to marked destruction of tissue, and since there is practically a specific treatment for this type of sore, we feel that attention should again be called to this condition.

Bataille and Berdal<sup>4</sup> as early as 1889 recognized a contagious form of balanitis under the title, "balano-posthite erosive circinée," but Scherber and Müller<sup>5</sup> in 1904 were the first to isolate and identify the causative organisms, and they further demonstrated that the erosive and gangrenous types were due to the same germs, differing merely in the severity of the pathologic process.

We are likewise indebted to them for the term "fourth venereal disease." As early as 1904 they reported fifty such cases, and in a subsequent most complete article published in 1910 Scherber<sup>6</sup> reported eighty-one cases found during a period of four years in Finger's Clinic at Vienna.

Corbus, in his communication published in 1913,<sup>2</sup> calls attention to articles concerning this disease published in England, Austria, Switzerland, Italy, and Greece. We have been unable to find any references to this condition published subsequently, except one in a South American journal which was not available for study.

### DEFINITION.

Corbus defines the disease as, "a specific infectious venereal disease due to symbiosis of a vibrio and a spirochete, with local and constitutional symptoms varying with the severity of the infection."

\*From the Research Department of the Detroit Clinical Laboratory.

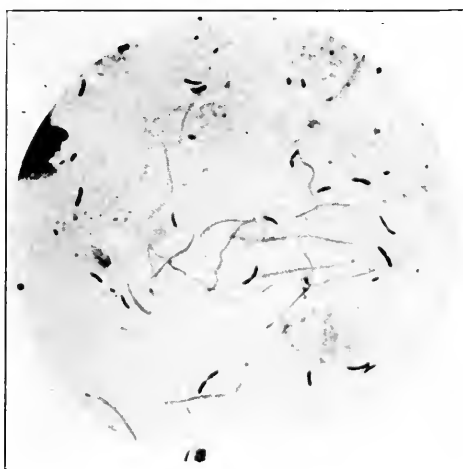
## BACTERIOLOGY.

The bacteriologic picture found in these cases is identical, on morphologic grounds, with that of Vincent's angina, ulceromembranous stomatitis, noma, and other ulcerative and gangrenous processes which affect the mucous membranes, or at least have their point of origin in these tissues.

Smears made from the discharge show fusiform bacilli or vibrios and spirochete forms.

The fusiform bacillus, or vibrio, is a weakly Gram-positive organism about 0.8 microns wide by 2 microns long, of a spindle shape, and shows one or more granules in the central portion. It shows no motility under the dark-field. This form can be grown on ascitic or serum agar under strict anaerobic conditions, where it appears in the form of short rods, at times filamentous in character.

The spirochete is rather thick as compared to the pallida and possesses a



Smear from case of erosive and gangrenous balanitis, showing fusiform bacilli and spirochetes. Magnification: 1200 diameters. Stained with dilute carbol-fuchsin.

very active motility, there being both a wave-like and a rotary movement. It is about 0.2 microns wide and varies in length from 5 to 15 microns. The spirals, of which there are usually from six to ten, are about  $\frac{3}{4}$  microns in width and are not as numerous nor as fine and regular as those observed in the *treponema pallidum*. Once seen under the dark-field illuminator, there should be no great difficulty in distinguishing between the two forms. This organism can also be cultivated anaerobically, but is much harder to isolate than is the accompanying fusiform bacillus.

Both the fusiform bacillus and the spirochete stain readily with dilute carbol-fuchsin, another point distinguishing the latter from the pallida. The spirochete does not retain the stain by the Gram method.

Hoffman and Prowazek<sup>7</sup> who gave the name of *S. balanitidis* to the organism described by Scherber, consider it a specific type distinct from those found in the mouth and also consider it as not being identical with *S. refringens* with

which it was classed by Levaditi<sup>8</sup> who grew *S. refringens* in collodion sacs intraperitoneally and endeavored unsuccessfully to produce balanitis with such cultures. He, therefore, concluded that this organism could not be considered as the cause of the disease; while Scherber, who was unable to inoculate animals with cultures of the vibrio, held that it was. On the other hand, Corbus thinks the vibrio form is the active agent since spirochetes similar morphologically, at least, to those observed in balanitis, may be found in the preputial sac under normal conditions.

Weaver and Tunnicliff<sup>9</sup> isolated in pure culture fusiform bacilli from Vincent's angina and noma and later Tunnicliff<sup>10</sup> found such organisms in normal mouths. She considers that the vibrio and the spirochete represent merely different forms of the same organism; but Ellermann<sup>11</sup> and Krumwiede and Pratt<sup>12</sup> believe them to be separate and distinct species. The latter observers cultivated vibrios from a number of different sources and found that regardless of origin, they could be divided into groups according to whether they fermented saccharose or not. Noguchi<sup>13</sup> considers that *S. balanitidis* "probably has a more intimate relation to the lesion than that of a mere secondary invader," a point strongly emphasized by Scherber's discovery of the organism not only in the initial lesion but also in the lumina of the adjacent blood vessels and in the inguinal lymph nodes.

The evidence at hand seems to prove that these symbiotic organisms, often present in the mouth without any manifestations of pathogenicity, may, under conditions of lowered vitality, disease, etc., develop malignant properties, and when transferred to other fields of endeavor produce ulceration and sloughing of a marked degree.

Corbus was able in all his cases to get a history of coitus in os or of wetting the genitals with saliva. We elicited such a history from two of our patients, while three others denied such exposure. The sixth patient vehemently denied exposure of any kind.

McDonagh<sup>14</sup> considers the condition as usually resulting from coitus in os and thinks that the woman need not necessarily have an acute vulvitis or vaginitis, as the mere presence of a few of these organisms may lead to their implantation on a glans penis, which, if covered with a long foreskin, furnishes ideal anaerobic conditions for the propagation of the species. Indeed, he even goes so far as to state that the infection need not necessarily come from the woman, mere mechanical irritation and inflammation at times instituting such a process. While these divergent views leave the exact relation of these organisms and their source of origin in doubt, nevertheless the fact remains that in certain cases of balanitis where a retracted foreskin furnishes anaerobic conditions, a symbiosis of certain anaerobic organisms is present. Moreover, these cases respond promptly to the removal of such anaerobic conditions and the administration of oxygen in the form of hydrogen peroxide. Such treatment is not effective with the true chancroidal sore.

#### SYMPTOMATOLOGY.

The disease usually begins on the sulcus coronarius, prepuce, or glans; usually as one, sometimes as several small superficial erosions which may heal

in the course of a few days without treatment. At times the surface of these ulcers may show a bright red color, while at other times they are covered with a grayish film of coagulated exudate and necrotic tissue. Where several small lesions are present, they usually coalesce in the course of a few days.

The most, in fact the only, characteristic symptom of this type of venereal sore is the early and abundant production of a white or yellowish pus of a most foul odor. This pus, however, may be lacking in the early stages of the disease or may have been kept in abeyance by mechanical cleansing.

The lymphatic glands are enlarged and usually painless, with the dorsal lymph cord sharing in the lymphatic involvement. Aside from the inguinal region there is no adenitis. Suppuration of the affected lymph glands does not occur.

The majority of patients show no constitutional symptoms, but in the gangrenous type where the destruction of tissue may be rapid and extensive, profound sepsis may supervene.

Phimosis and swelling appear early and are often marked, making treatment difficult without surgical intervention.

#### DIFFERENTIAL DIAGNOSIS.

This type of sore is most apt to be confused with chancroid, as both have the same incubation period, the sore usually appearing within two to seven days after exposure.

In chancroid, however, the ulcers are more likely to be multiple, and show contact infection on apposing surfaces which we have not observed in balanitis; and there is much greater tendency manifest to undermining of the edges of the ulcer, with the production of a ragged and irregular border. Painful inguinal adenitis with resulting bubo is common in chancroid, but has not been observed in balanitis of this type.

Balanitis cases show more abundant pus of a foul odor, and produce earlier and more marked edema and swelling. Phimosis is very common and appears quite early.

The process is differentiated from the Hunterian chancre by the much shorter incubation period, by the rapidity of development of the pathologic process and the production of the pus described above.

However, we have long ceased to depend on visual observations alone in differentiating between chancre and chancroid; for while there is a typical hard chancre, it is so often masked by an accompanying chancroidal infection, that diagnosis is difficult if not impossible.

Indeed, we have found that at least 30 per cent of our cases of true syphilis are complicated by a chancroidal infection and such cases can be diagnosed with certainty only by means of microscopic and serologic methods.

#### TREATMENT.

The organisms are strictly anaerobic, and consequently can not exist except where a long foreskin provides such conditions.

In early cases before phimosis has become marked, retraction of the fore-

skin with mechanical cleansing and the application of hydrogen peroxide furnish an oxygen supply incompatible with the growth of these organisms. We have had our cases apply gauze soaked in ordinary commercial hydrogen peroxide, covered with oiled silk, after careful mechanical cleansing of the ulcer and the removal of all sloughing tissue.

Where this strength of peroxide tends to be irritant it can be used in a diluted form.

If the phimosis be marked or if the foreskin can not be kept retracted, dorsal incision should be made at once owing to the rapid advance and marked destructive action of the process if unchecked.

It is neither necessary nor advisable to burn these sores, because burning alone, unless aerobic conditions are maintained, will have but little effect on the process. Neither should carbolic acid or other strong disinfectants be employed, as any chemical causing tissue destruction simply furnishes more ideal conditions for the growth of these organisms.

As with Vincent's angina, salvarsan or neosalvarsan produces a prompt cure, while these drugs have no beneficial action in chancroidal infections. However, in general, the local treatment advocated is all that is necessary. Should the process be unchecked by hydrogen peroxide, or if gangrene be already manifest, resort should be had to these remedies, in conjunction with the local treatment with peroxide.

#### CASE REPORTS.

Our six cases were examined within one to three days after the sore appeared, all being in the erosive stage, with an abundant discharge of foul pus, moderate edema with slight phimosis, and exquisitely tender ulcers, the largest sore being about the size of a dime. The ulcers were covered with pus and considerable coagulated serum and exudate, beneath which lay a very superficial reddish-colored erosion. All showed moderate inguinal adenitis, painless in character. There were no constitutional symptoms. Two patients gave a history of unnatural intercourse, the other four denied it. In all of these cases the sore had appeared within four days after exposure.

One patient with a history of repeated exposures, but denying coitus in os showed *treponema pallida*, as well as the organisms characteristic of this condition. This case cleared up promptly under salvarsan and local treatment with hydrogen peroxide.

Three other cases treated with wet dressings of hydrogen peroxide cleared up within a few days.

The remaining two patients were advised to use the same treatment and presumably cleared, as they failed to return to their attending physician.

None of these cases showed the presence of the Ducrey bacillus.

#### CONCLUSION.

1. There is a type of venereal sore due to symbiotic action of a vibrio (*fusiform bacillus*) and a spirochete.

2. The lesion may be simply erosive in character, or show marked sloughing and gangrene.

3. These organisms are probably of oral origin, and are closely allied, if not identical, with the organisms of Vincent's angina.

4. Hydrogen peroxide acts as a specific in this type of venereal infection.

5. Cauterizing and strong antiseptics should not be used as they tend to produce necrosis of tissue, thus favoring the further growth of these organisms.

6. The gangrenous type demand salvarsan or neosalvarsan, as well as local treatment.

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## A POSSIBLE EXPLANATION FOR THE CYANOSIS AND HYPERPNEA SEEN IN PNEUMONIA AND CARDIAC DECOMPENSATION\*

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IN normal individuals the minute volume of the respired air is primarily determined by the amount of carbon dioxide, and secondarily by the amount of oxygen present in the blood coursing through the respiratory center. Lack of oxygen is a much less effective stimulus to the center than is a corresponding increase in the carbon dioxide of the blood. An impairment in the gas exchange in the lung so that the blood leaves the heart with too little oxygen and too much carbon dioxide will cause the blood to be dark or cyanotic, and the respiratory center to increase the minute volume of the respired air. This results in a lowering of the percentage of the carbon dioxide in the alveolar air or its concentration in the arterial blood. Whether the oxygen content is affected will depend upon the nature of the respiratory lesion, as will be pointed out later. This type of cyanosis is primarily arterial, and the hyperpnea is caused by too much carbon dioxide in the arterial blood. We believe that this type is present in cases of pneumonia that are cyanotic without evidence of air hunger or cardiac impairment, and that it is essentially like that found in congenital heart disease.

In cardiac decompensation without respiratory impairment, the minute volume of the blood circulating through the lungs and the respiratory center is

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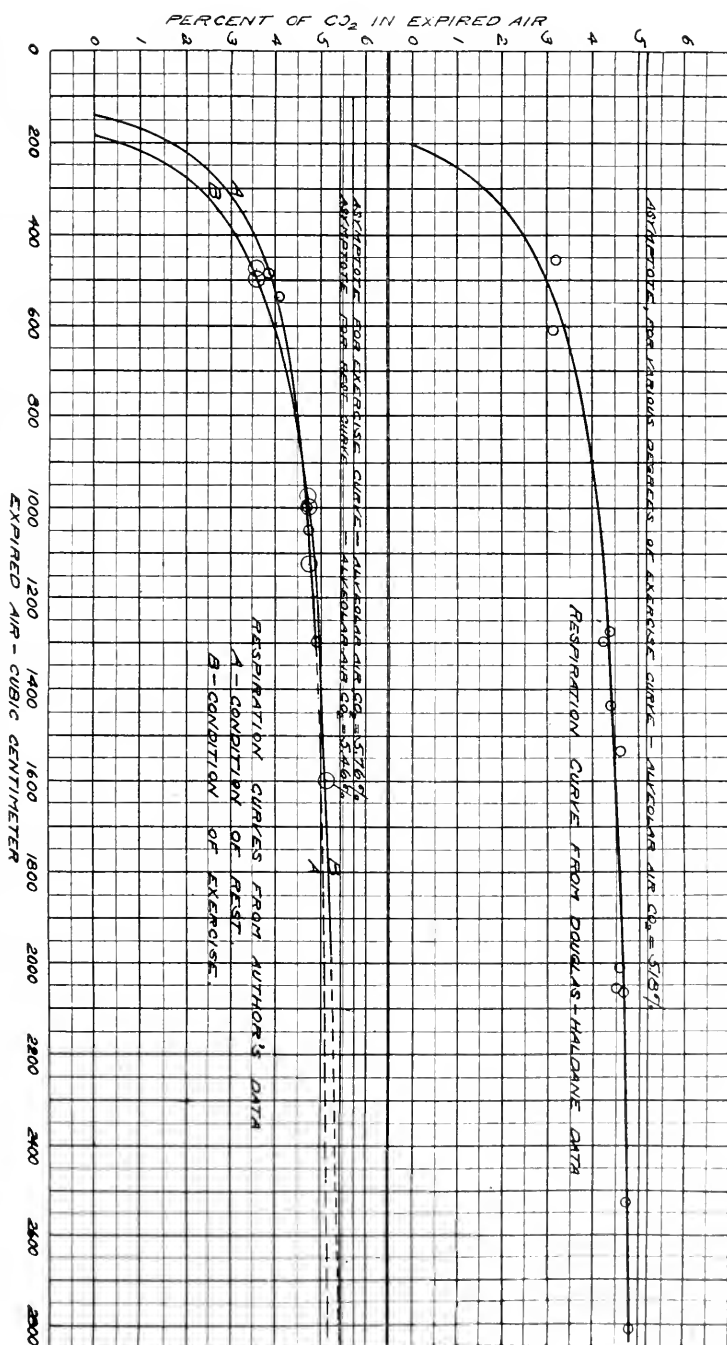
diminished and, if metabolism is normal, the blood per unit volume must carry more oxygen to the tissues and more carbon dioxide from the tissues than under normal conditions. If the oxygen supply is greatly diminished, the excitability of the respiratory center is increased and, in case the blood supply is not sufficient to promptly carry away the carbon dioxide produced, the center is abnormally stimulated. The compensating mechanism, brought about by increasing the minute volume of air respired, is the lowering of the carbon dioxide in the arterial blood entering the center to such a degree that the blood as it passes through the center is able, in spite of its small volume, to carry away the carbon dioxide formed without increasing the carbon dioxide tension in the center. The oxygen content of the venous blood, however, is much less than under normal conditions, inasmuch as the hemoglobin has been compelled to give up a greater percentage of its oxygen. The venous blood is therefore dark or cyanotic. Peabody<sup>1</sup> has shown that the respiratory center of patients suffering with cardiac decompensation is much more sensitive than normally to changes in the carbon dioxide tension of the alveolar air.

Ordinarily the alveolar air has the same pressure of carbon dioxide as does arterial blood, and the determination of this factor in either will give its value in the other. The direct relationship between the combined and the free carbonates of the blood and the tension of carbon dioxide in the alveolar air in normal individuals is well established. For this reason the method devised by Van Slyke<sup>2</sup> for the determination of the total blood carbonates is capable of giving fairly accurately the tension of carbon dioxide in the alveolar air as determined by various methods.

Walker and Frothingham<sup>3</sup> have published a rather extensive series of cases in which they compared the carbon dioxide in the alveolar air as determined by the Plesch method, with the carbon dioxide in the venous blood as determined by the method of Van Slyke. They conclude that the two methods give approximately the same results, and found the most marked variations in their cases of pneumonia and Graves' disease. In these diseases, because of the increased rate of metabolism Plesch's method undoubtedly gives too high results.

The methods for determining the percentage of carbon dioxide in the alveolar air are not very satisfactory for accurate clinical use. Haldane and Priestley's method,<sup>4</sup> while it has yielded most valuable data in the physiology of the regulation of the respiration, is not a satisfactory clinical method, and does not give very accurate figures in all conditions. By making use of the mathematical laws of vanishing small quantities, I have devised a method for the determination of the dead space and the percentage gaseous composition of the alveolar air.<sup>5</sup> I have used this principle for a year, and have only recently discovered that Professor Almeida, of the University of Rio Janeiro, has published a preliminary announcement of a method for the determination of the carbon dioxide in the alveolar air which is based on the principle I have used. Priority of discovery, therefore, belongs to him. Briefly the principle of the method depends on the influence which the atmospheric air of the dead space has in diluting the gases of the alveolar air in the total expired air. The principle can be shown graphically by the use of a curve, the abscissas of which represent the amount of the expiration in cubic centimeters, and the ordinates the percentage of carbon dioxide or oxygen in the expired sample. The larger the expiration, the greater





Upper Figure.—Curve constructed from data taken from experiments by Douglas and Halldane, in which the depth of breathing was determined by the amount of muscular exercise. (four. Physiol., 1914, 45, 235.)

Lower Figure.—Curve A gives data obtained at rest; B, when author is walking three and a half miles per hour. (Am. Jour. Physiol., 1917, xliii, 73.)

percentage of carbon dioxide does the air contain and the less percentage of oxygen, because the diluting factor which the dead space adds becomes less and less important as the expiration increases in amount. By making several observations in which the expirations are progressively increased, it will be seen that the percentage of either the carbon dioxide or the oxygen, when plotted on cross-section paper, gives a perfect hyperbolic curve in which two constants are

present. One is the percentage of carbon dioxide in the alveolar air and is represented by the horizontal asymptote, and the other the capacity of the dead space, which is represented by the vertical asymptote.

The percentage of carbon dioxide in the alveolar air as determined by this method is a trifle lower than that obtained by other methods in use at the present time. However, it is more accurate because it has not the theoretical errors present in other methods and, although involving a greater time and more complicated apparatus to carry out, it requires less cooperation on the part of the patient than do the other methods.

In the Lakeside Medical Service we have been using this method to determine the percentage of carbon dioxide in the alveolar air, in company with Van Slyke's method for the determination of the blood carbonates, in a number of cases of pneumonia and cardiorenal disease. Our series is not large enough to warrant a complete report, but the results so far obtained throw some new light on the pathologic physiology of these diseases.

In several cases of pneumonia in which cyanosis was present without signs of cardiac impairment or air hunger, we have noted that the carbon dioxide tension of the alveolar air as determined by our method is decidedly lower than that obtained by Van Slyke's method. In the greater number of pneumonias seen late in the disease and without cardiac impairment, we found that the blood carbonates and the alveolar air carbon dioxide determinations by the two methods corresponded fairly well. Cyanosis in these cases was never marked.

Especially interesting is one case whom I saw twenty-four hours after the onset of the attack. His right base was dull, with high-pitched bronchial breathing. His respirations were 28 to the minute, pulse 115, and temperature 104°. The man appeared very ill, but was able to cooperate with me and was fairly comfortable. He had no signs of cardiac impairment, and the blood pressure was 135 systolic and 85 diastolic. The most noticeable thing about him was the intense *cyanosis*, which was present without symptoms of respiratory distress.

Examination of his blood by the Van Slyke method showed practically a normal blood carbonate reserve, and the alveolar air as estimated by this method gave 37.5 mm. Hg. carbon dioxide pressure. His alveolar carbon dioxide determined by my method was 26 mm. The following day his general condition was much worse. The right upper was consolidated, his temperature 105°, pulse 140, and his blood pressure a little lower. His cyanosis had disappeared. He was irrational, and I could not obtain a sample of air for carbon dioxide determination of the alveolar air. His blood carbonates were, however, the same as the day before. We believe that an explanation of the cyanosis and the disappearance of it as the patient grew worse may be found in a consideration of the respiratory physiology of congenital heart disease.

Last year Dr. Julius Hess and I<sup>6</sup> reported three cases of congenital heart disease occurring in boys of the same family. In all we found a low percentage of carbon dioxide in the alveolar air, an observation which has been found by others in congenital heart disease. The blood carbonates by Van Slyke's method and the venous carbon dioxide tension by the method of Christiansen, Douglas and Haldane<sup>7</sup> were normal. We are, therefore, forced to conclude that the blood which enters the lungs of these boys is more completely freed of carbon dioxide than in the normal individual, and that less than the normal amount of blood is

passing through the lungs. The blood leaving the lungs, therefore, carries a full load of oxygen and less than the normal amount of carbon dioxide. On reaching the left heart it mixes with blood coming directly from the right heart through the abnormal connection. This blood is venous blood having a high carbon dioxide and a low oxygen content. The result of the mixture gives a blood probably almost normal with reference to the carbon dioxide content, but less than normal with reference to its oxygen content. The boys all have an increased minute volume of respired air, but do not show any evidence of air hunger, nor is their capacity for work much less than that of the normal controls.

We interpreted these findings as indicating that these boys have potentially a condition of carbon dioxide acidosis, which they compensate for by over-ventilating the blood which passes through the lungs so that the blood which leaves the left heart may have a normal carbon dioxide tension. In the case of oxygen, however, since the blood leaving the lungs carries all the oxygen it can carry, no compensation can take place, and the mixture of blood in the arterial system is unsaturated with oxygen and hence blue in color.

A very similar explanation can be given for the cyanosis which we have observed in pneumonia. Let us assume that the pathologic process which destroys the respiratory membrane and makes the exchange of gases impossible in the pneumonic lung precedes the closing of the pulmonary vessels. Such a condition will give physiologically the same conditions as are present in congenital heart disease. The blood reaching the left ventricle would in this case be a mixture of venous blood from the diseased area, and arterial blood from the sound area of the lungs. The respiratory center, responding to the increase of the carbon dioxide content of the blood, increases the minute volume of air respired and lowers the carbon dioxide tension in the arterial blood. This would tend to make the carbon dioxide concentration of the blood normal, but the oxygen content would be determined by the relative amounts of venous and arterial blood being delivered to the left ventricle. It would always be less than normal, since there is no possible mechanism to increase materially the amount of oxygen which the blood will take up. Such a condition can exist without evidence of air hunger or respiratory distress so long as the oxygen supply is sufficient. However, cyanosis would be present, since the capillary blood is not saturated with oxygen. As the disease progresses, the circulation through the involved lungs becomes less and less, and the cyanosis disappears.

We find confirming evidence of this view in the studies which Porter and Newburgh<sup>6</sup> have made on the condition of the respiratory center in pneumonia. These investigators find the respiratory center is depressed through a peripheral afferent vagus mechanism. The pneumonic respiratory center, being less sensitive to an increase in the carbon dioxide content of the blood than is the normal one, requires a larger amount of carbon dioxide in the arterial blood for the effective stimulation of the center. We have found confirmatory proof of this view in the case of one cyanotic patient, in whom we found the alveolar carbon dioxide and the blood carbonates to be practically normal, but whose venous blood, as determined by the method of Christiansen, Douglas and Haldane<sup>7</sup>, was higher than normal. This condition indicates that the amount of blood passing over the respiratory membrane was reduced, and that the blood entering and leaving the respiratory center had a higher content of carbon dioxide than under

ordinary conditions. This offers an explanation for the fact that many cases of pneumonia, which are cyanotic because they are receiving unrespired blood in their left heart, fail to show much if any decrease in the percentage of carbon dioxide in the alveolar air. That it is found in some cases, however, is significant of its potential existence in all. In other words, were it not for the hypesthesia of the respiratory center which Porter and Newburgh find present in pneumonia, we should probably more often find the alveolar carbon dioxide lower than the blood carbonates indicate. Butterfield and Peabody<sup>9</sup> have furnished some experimental evidence that cyanosis of pneumonia may be caused by the formation of methemoglobin. I do not wish to deny that this occurs, but I believe that the degree of cyanosis which we see developing early in the disease is out of all proportion to the infection. Moreover, the blood of these cyanotic patients when shaken in the air, assumes a very arterial color, which is in striking contrast to the deep dark color which persists in the blood of patients who have been poisoned with coal tar products. One can not explain the sudden disappearance of the cyanosis in the cases mentioned on the theory of methemoglobin formation. It appears probable to us that the cyanosis disappeared when the amount of unrespired blood which was reaching the left auricle became negligible.

We believe that in these cases of pneumonia which we have described, one portion of the blood circulating through the pulmonary vessels consists of blood which has been superventilated, while another portion which passes through the diseased area is unventilated. Therefore, the confluence of the total blood in the left auricle gives a mixture of superventilated blood with unrespired blood. This will give a total result of an aortic blood which will have a fairly normal amount of carbon dioxide but a low amount of oxygen. The lack of oxygen is responsible for the cyanosis.

In several cases of cardiac disease we have found the percentage of carbon dioxide in the alveolar air low during the stage of cardiac decompensation. In these cases there was no lowering of the total blood carbonates, and as the heart compensated by rest in bed and treatment, the alveolar air carbon dioxide and the blood carbonates came back into agreement. Recently, with Dr. Christie, I have had one case of especial interest. The patient was admitted to the ward with a badly decompensated heart, air hunger, orthopnea, high systolic and diastolic blood pressure, and a dilated heart. His blood carbonates gave a normal figure, indicating no reduction in his blood alkalinity. His minute volume of respired air was 10 liters, and his alveolar air determined directly gave a carbon dioxide pressure of 30.7 mm. Hg. His blood failed to show evidence of acidosis, while his alveolar air indicated that it was present. His venous carbon dioxide pressure, as determined by the method of Christiansen, Douglas and Haldane, was about 46 mm. Hg. The wide difference between the pressure of carbon dioxide in his arterial and his venous blood indicates that the blood which was passing through his lungs was being superventilated. In this patient the blood entered the lungs bearing approximately the normal load of carbon dioxide, but left the lung with a less than normal amount of carbon dioxide. It entered with a carbon dioxide pressure of 46 mm. and left with a carbon dioxide pressure of 30. Since his blood was approximately normal in its capacity to carry carbon dioxide, we may compute from the above data, together with a determination of his total respiratory exchange, the minute blood flow through his

lungs from the data furnished by Christiansen, Douglas and Haldane on the relationship between the oxygen and carbon dioxide content of the blood. I have done this and find that each 100 c.c. of blood which passed through this man's lung lost at least 10 c.c. of carbon dioxide. The normal figure given by most authorities is about 6 c.c. This figure, taken together with the total carbon dioxide eliminated by the patient per minute, which in this case was a normal figure, gives him a minute cardiac output of about 3 liters per minute, which is about one-half the calculated normal figure. Because of the small amount of blood which passes through the respiratory center, the metabolism of the respiratory center itself would cause the tension of carbon dioxide to be raised above a normal level, unless the blood entering it is capable of carrying away more carbon dioxide than it does under normal conditions.

Lewis<sup>10</sup> observed similar cases, and Peabody<sup>1</sup> has reported a series of cases which undoubtedly fall into this group. Lewis believes that the cyanosis and dyspnea without acidosis are due to poor oxygenation of the tissues. Peabody, basing his conclusions on the low alveolar carbon dioxide pressure in these cases, believes they have a real acidosis. He, however, recognizes the part which the accumulation of carbon dioxide in the respiratory center may play in the dyspnea. Recently Peters reported similar cases, but attributes them to a failure in the gas exchange in the lung. He did not, however, determine the tension of the carbon dioxide in the venous blood. Such an explanation can hardly apply in our case for the following reasons; the respiratory exchange and the tension of the carbon dioxide in the venous blood was nearly the same during and following the stage of decompensation. Under these conditions, if the blood leaving the lungs contained a normal, or even slightly greater than normal, tension of carbon dioxide the mass movement of the blood would have to be normal unless the tension of the carbon dioxide in the venous blood was raised. There can be no doubt that the mass movement of blood in our case was seriously impaired. From both a physical and a physiologic standpoint we believe our explanation is more in accord with the facts than that given by Peters, but he is quite right in believing that these cases are examples of carbon dioxide acidosis.

From the above results we see that the percentage of carbon dioxide in the alveolar air is not a good guide as to blood acidosis, at least in cardiac disease, pneumonia and congenital heart disease. In the cardiac case a low carbon dioxide alveolar air pressure is a result of the attempt of the respiratory center to prevent its venous carbon dioxide pressure from being unduly raised because of the slow circulation. In pneumonia and congenital heart disease, the respiratory center reacts to keep the arterial blood at a normal carbon dioxide tension.

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- <sup>9</sup>Jour. Exper. Med., 1913, xvii, 97.
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## EXPERIMENTAL ASTHMA IN THE GUINEA PIG

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THE material used by the body in its metabolism enters from without through definite channels, the alimentary canal and the respiratory cul-de-sac.

The great advances in our knowledge of nutrition have been made possible through a better understanding of digestive proteolysis. Hitherto the respiratory epithelium in its relation to nutrition has engaged physiologists chiefly as the seat of alveolar gas exchanges. But in stepping across the "no man's land" from physiology to pathology the epithelium of the upper air passages acquires unrivaled importance in its relation to foreign proteins inhaled with the air and which, when absorbed, cause profound metabolic reactions and a large proportion of the disease of mankind. The current statements<sup>1</sup> that in healthy man the mucous membrane of the air passages, except at the anterior end of the nasal canal, is sterile are extremely significant of active digestive processes carried out under its control. On the contrary, the wealth of bacteria found in the secretions of respiratory disease suggests that the epithelium has lost destructive power over germs and that this failure is a concomitant of, if not a necessary factor in, the development of disease.

We seem, unfortunately, to be absolutely ignorant of the local reactions between protein substances and the respiratory epithelium upon which they are implanted and, in view of certain literature, it is worth while to repeat the truism that advance of knowledge in this field can be expected through experiment only, however important may be the aid of imagination.

Without understanding in the least the reactive attributes of the mucous membrane of the nose, it is easy to apply a foreign protein to this surface and afterwards elicit, through the reactions of immunity, signs of its absorption.

It seems reasonable to assume that an understanding of at least those diseases which are due to invasion through the respiratory tract should be most readily attained by introducing into the body the experimental reagents through the same avenue as that traversed by the disease-producing material.

It was considerations such as these which led me several years ago to submit to study the immunologic reactions set up in guinea pigs by the application of foreign proteins, normal horse serum, to the mucous membrane of the nose.

Generalized reactions such as those to be described evidently are similar in pathology to such as have recently been made familiar by numerous investigations into the so-called "hay fever."

The epoch-making work of Dunbar<sup>2</sup> clearly demonstrated the anaphylactic character of the hay fever phenomena. He probably makes a fundamental error in ascribing the susceptibility of hay fever patients to attacks of pollinosis to an abnormal permeability of mucous membranes and skin in such subjects, while immunity from the disorder is supposed to depend upon the successful resistance of the epithelium to absorption of the pollen proteins. My work with Powell<sup>3</sup> on guinea pigs indicates that proteins of horse serum are absorbed by

the mucous membrane of the nose with every application of the serum and definitely proves that as few as six instillations of serum in alternate nostrils to the amount of 0.19 c.c. produces a systemic effect in every case. These effects were pronounced in high degree when six instillations were given at intervals of twenty-four days, each nostril receiving its three doses at intervals of forty-eight days.

In our experiments the systemic action of the nasal instillation was measured by the reactions induced by the intravenous injection of horse serum. The somatic change caused by the instillation of serum was of two varieties. The animal was rendered hypersensitive to the serum, so that an intravenous injection of 0.25 c.c. would cause death within 3 to 5 minutes, or produce positive reactions of every possible grade of intensity. Or there would be little or no perceptible anaphylactic disturbance, and later a succession of intravenous injections at wide intervals would be withstood, showing that active immunity had been established. We were able to satisfy ourselves by further work<sup>4</sup> that the prominence of the anaphylactic symptoms in the process of immunization depended upon the amount and period of contact of the serum instilled with the mucous membrane of the nose, that is, probably, upon the amount of serum absorbed. Using minimal quantities of serum (0.04 c.c.) for the instillations, a high degree of immunity towards horse serum could be imparted, with nearly complete elimination of the symptoms of hypersensitiveness when the guinea pig was given a considerable intravenous injection of the antigen.

Transferring these general results to the special case of hay fever, the conclusion is plausible that some degree of absorption of pollen protein is universal among people exposed to pollen; that most people become immune to pollinosis because of the minimal initial absorption, but that in subjects of hay fever a large initial dosage has set up a hypersensitiveness towards pollen which the tissues of the body always tend to retain. An exact analogy is to be found in the experiments to be presently detailed.

Practical work on this subject impresses one with the distinction between general and local hypersensitiveness. Whenever a normal guinea pig instilled with serum into the nose develops a respiratory reaction, such as the production of rales, that animal always succumbs to the intravenous injection of serum, but, as will be shown later, animals which react to intranasal serum by the most violent asthma can, by appropriate treatment, be made temporarily immune to respiratory disturbance, though they quickly succumb to intravenous injection of serum.

Our observations indicate that normal guinea pigs, no less than those that have been sensitized by subcutaneous injection of serum, can be made to develop attacks of asthma by instillation of horse serum into the nose; but in the latter, previously sensitized, animals the reactions are apt to be more violent and the latent period of local sensitization is decidedly shorter.

In a former article<sup>5</sup> I have described in detail the reactions to be expected when a normal guinea pig is treated with horse serum by the nose. With the animal held gently on its back upon a table, the head well extended, small droplets of serum were allowed to fall slowly from a hypodermic syringe upon one or the other of its nostrils. A variable amount of resistance in the way of strug-

gles and expulsive movements was encountered; a common reaction, more prominent with the progress of sensitization, was an excessive secretion of saliva. One of the most characteristic reactions to the instillation of serum in sensitized animals is an increase in intestinal peristalsis. In pregnant females uterine, and apparently independent fetal, movements are pronounced and may be extremely violent while the mother lies quite placid. Such reactions come on within one to three minutes after beginning of instillation. My usual method was to instill 0.1 to 0.2 c.c. horse serum at intervals of one to four or more days, alternate nostrils being instilled at successive treatments. In a small proportion, possibly one-fifth of the normal animals receiving this spaced treatment, there would develop, with the instillation given about the sixteenth day, moist rales, apparently beginning in the nose and extending through the trachea to the bronchi. At the same time a more or less pronounced dyspnea supervened, the respiration usually being slowed, but sometimes quickened. The phenomena are so suggestive of those of bronchial asthma in man that there can be little chance of error in considering these attacks true bronchial asthma. These asthmatic paroxysms may develop under conditions most unfavorable for the development of anaphylactic demonstrations, namely, in the course of a series of instillations separated by intervals far shorter than the period of incubation. This fact alone indicates a qualitative difference between the immunologic reactions which attend nasal as contrasted with subcutaneous inoculations. When, on the other hand, a normal guinea pig is caused to absorb serum through the nose by giving it three or four instillations on successive days and then is allowed to rest, a final instillation after twelve to sixteen days as a rule provokes at least the initial signs of asthma.

Even a single instillation of horse serum produces a systemic effect, for if it is repeated after a sufficient interval unmistakable signs of sensitization may be disclosed. A group of eight normal guinea pigs was instilled each with three minims of horse serum into the right nostril. They were divided into four groups of two pigs each and the instillation was repeated, but now into the left nostril, at the end of six, nine, twelve and seventeen days. There was no certain anaphylactic reaction at the second instillation in the first two groups. But in the pigs treated after twelve days a few tracheal rales developed, and the same result to a more marked degree attended the instillation after seventeen days' rest.

In another experiment, two pigs received by the nose 0.25 c.c. horse serum. Sixteen days later the treatment was repeated with the production of slight anaphylactic symptoms such as excessive secretion of saliva and rubbing of the nose. Two days later one of these animals received an intravenous injection of serum. There was no reaction, though the animal succumbed to a subsequent injection. The important conclusion is suggested that sensitization of the peripheral mechanism may reach a fairly high degree through a single instillation of serum while there is as yet no general hypersensitiveness demonstrable by intravenous injection of the antigen.

In a group of three pigs which had received a course of nasal instillations followed by several widely-spaced intravenous injections of serum and which, accordingly, had developed a high degree of immunity towards serum, a nasal in-



stillation of 0.25 c.c. in each, given after a resting period of several months, provoked no response. The most probable explanation of the result is that the general immunity had enveloped the respiratory apparatus.

It may be well to repeat here an observation made in another place,<sup>4</sup> that the evidence shows that states of sensitization or immunization set up by a few intranasal instillations of serum are of short duration, lasting fifty to seventy-five days, while corresponding conditions induced or strengthened by relatively large doses administered parenterally are apparently permanent.

We have seen above, evidence, supported by a great number of additional experiments, that every intranasal instillation of serum in normal guinea pigs is attended with some absorption and that after a single such absorption a repetition of the treatment in twelve to seventeen days leads usually to reactions indicating hypersensitiveness of the respiratory apparatus.

When the intranasal treatment is carried out on guinea pigs which have, weeks previously, been made hypersensitive to horse serum by subcutaneous injection there is more or less quantitative modification of the respiratory reactions. General sensitization does not, as a rule, induce hypersensitiveness of the respiratory apparatus to horse serum applied to the mucous membrane of the nose.

The nasal mucous membrane must apparently be independently sensitized, but the latent period of its sensitization may be materially shortened. Only in one out of scores of animals tested (pig 2 of the table) was asthma induced by the very first instillation of serum into the nose. It is not uncommon, however, for the latent period to be shortened to from one to five days.

A subcutaneously sensitized pig, when given intranasal treatment, usually responds clearly to a second instillation given about eight days after the first. The anaphylactic reactions to nasal instillation are usually more severe in animals previously generally sensitized than in those termed normal.

The fact that a pig previously sensitized by subcutaneous injection rarely responds to nasal instillation of serum until several treatments have been given, indicates a progressive change in the nasal epithelium which may be of the nature of "sensitization" or merely the sign of an increased permeability. Such an animal responds with asthma to instillations repeated daily more readily than when a wider interval of rest is chosen. It is as if the closer succession of instillations found the epithelium in Wright's "negative phase."

If one may venture to draw a parallel between serum anaphylaxis and that of hay fever, I can not subscribe completely to the views recently expressed by Cooke, Flood and Coca, that "hay fever is the clinical symptomatic expression of local hypersensitiveness."<sup>6</sup> I have found no extreme local hypersensitiveness without a background of general hypersensitiveness.

There would seem to be a marked difference between the conditions of local anaphylaxis in the mucous membrane of the nose and of the skin in their relations to general sensitization.

Observers experienced in the skin tests for general hypersensitiveness seem to regard the first scratch to be as diagnostic of the general condition as subsequent ones.

Reactions of the hypersensitive respiratory apparatus to the introduction of horse serum into the air passages are complex events. The most measurable

anaphylactic symptom is that of excessive secretion leading to the production of rales which apparently begin in the nose and soon extend through the trachea to the bronchi. With this disturbance there is associated more or less dyspnea. The dyspnea is evidently occasioned by an event other than the mechanical obstruction offered by the bronchial secretion; it is often most intense when the rales diminish, and auscultation under these conditions indicates an obstruction to the passage of air into the alveoli. In a paper cited,<sup>3</sup> attention was called to the fact that when the anaphylactic reaction was brought on by making the guinea pig inhale the serum in a finely vaporized cloud through a funnel, the respiratory rales did not develop but the dyspnea was often intense; the two methods of administering the serum produced in the one case a dry and in the other a moist asthma. It is obvious that the bronchial obstruction occurring independently of secretory accumulation is due to the phenomenon discovered by Auer and Lewis,<sup>7</sup> stenosis of the small bronchi which, according to these authors, is caused by spasm of the muscle fibers, encircling the tubes.

The writer suspects that edema of the cells lining the bronchioles forms a prominent if not a determining factor in the stenosis, but it is not yet prepared to assert this position.

The important outcome of the observations is the demonstration of an experimental condition, which has all the features of bronchial asthma, whose origin is undoubtedly anaphylactic.

It would seem obvious that there is parallelism if not identity between the pathology of experimental bronchial stenosis and that causing the paroxysms of certain forms of asthma, but the probably anaphylactic nature of the seizures was not apprehended until pointed out by Meltze.<sup>8</sup>

A considerable series of researches on the etiology of hay fever, among which should be mentioned specifically those of Goodale,<sup>9</sup> Koessler,<sup>10</sup> and, as regards asthma, Walker<sup>11</sup> were indispensable and admirable, but suffered from the limitations of observations confined to human beings. Those observers who have used the nasal route in administering the antigen to patients have found no advantage in it. This, I conceive, is due to the misapprehension that antigen so applied must necessarily provoke symptoms if absorbed. Only by animal experimentation can we expect to unravel the fluctuating relations between the exciting antigen and the sensitized organism, or deduce the laws according to which hypersensitiveness can be produced at will. The experiments to be described were conducted through a period of about thirteen months and were performed upon a group of guinea pigs sensitized to horse serum by subcutaneous or intraperitoneal injection, and were designed to throw light upon the following questions: 1. Can such animals be caused, in every case, to develop paroxysms of asthma by instillation of horse serum into the nose? 2. How may these paroxysms be modified by varying the intervals between the instillations and the amount of serum exhibited? 3. Is it possible to prevent anaphylactic seizures by doses of serum too small to cause them? 4. Can an "immunity" be developed against experimental asthma? 5. If local immunity is obtained, does it involve a loss of general sensitization?

#### METHODS AND RESULTS.

Only six guinea pigs were employed in these observations, which can only be regarded as a basis for further work. The time and amount of each dose of

serum instilled, and the reactions thereto are recorded in the table. The history of each animal preliminary to beginning the instillations was briefly as follows:

G.P. I.—Born October 26, 1915, of a mother which after a course of nasal instillations of serum had withstood three intravenous injections in the course of three months, the last injection of 0.75 c.c. being given, with very slight reaction, two days before birth of G.P. I. This pig when 15 days old, received a subcutaneous injection of 0.1 c.c. horse serum. The first instillation of the present series of experiments was given when the pig was 162 days old.

G.P. II.—Likewise offspring of an immunized mother which had received five intravenous injections in five months, the last two of 0.75 c.c. each, the last 27 days before the birth of G.P. II. G.P. II received 0.1 c.c. serum subcutaneously when 15 days old. First treatment of the series when pig was 121 days old.

G.P. III.—From the same mother as G.P. I, born about 82 days later. Received 0.1 c.c. serum subcutaneously when 25 days old. Born 84 days after mother had received last intravenous injection of 0.75 c.c. Was 80 days old at first instillation of this series.

G.P. IV.—Same mother as G.P. II but born later, 94 days after mother's fifth intravenous injection. Received subcutaneously 0.1 c.c. horse serum when 13 days old. Began instillation treatment when 80 days old.

G.P. V.—Born of *normal* mother. When about 60 days old received on four successive days intraperitoneal and one subcutaneous injection of horse serum totaling 12 c.c. Nasal treatments begun when about 115 days old.

G.P. VI.—From one of the serum immunized mothers described above but undetermined which. Gave subcutaneously 0.1 c.c. horse serum when about 69 days old and began the course of nasal instillations *on the same day*.

#### DESCRIPTION OF TABLE.

The whole course and outcome of the experiments is represented in the accompanying table.

The instillations (with Cutter's normal horse serum) were exhibited in two dosages. The smaller dose, two droplets from the hypodermic needle, represented about 0.04 c.c. of serum. The larger dose was a measured quantity of 0.2 c.c.

In the graphic record each cross on the abscissa line represents one day, and the numerals between the crosses indicate a corresponding number of resting days between successive treatments. The black crosses represent the instillations of smaller quantity, 0.4 c.c.; the red crosses, those of larger quantity.

A single cross signifies that no biologic reaction attended the administration of serum. An interrogation mark above it indicates that while no asthmatic attack developed, there were slight and obscure signs of anaphylaxis. The axis of ordinates contains the evidences of biologic reaction. Two crosses, one above the other, indicate a slight but obvious degree of asthma, three crosses much more pronounced or moderate asthma, and four crosses signify a very severe seizure such as, at times, seemed to threaten life. To illustrate by a definite example, in the case of G.P. V, three instillations of 0.2 c.c. serum were given on successive days. With the second instillation there was developed a slight asthma. On the fourth day there was given a small instillation of 0.04 c.c. followed by one day of rest when the instillation was repeated, provoking slight evidence of sensitization. After four small instillations on alternate days, a fourth large instillation was given resulting in a severe attack of asthma. The times taken to administer the dosages were, one and a half minutes for the small and three minutes for the larger instillations. Asthmatic symptoms fre-

quently developed during the act of instillation but usually within a minute or two after its completion. Their onset was seldom deferred more than fifteen minutes after administering serum.

#### REVIEW OF THE TABLE OF EXPERIMENTS.

Guinea pigs I and III should be considered together, since each seemed to possess unusual constitutional resistance against the sensitizing effect of serum and, after sensitization was secured, each developed a strong resistance against the exciting influence of nasal instillations and apparently a complete immunity to the induction of asthma. Both pigs were born of the same mother, but in different litters.

Each animal was observed through a period of 277 days during which guinea pig I received 21 large instillations. This animal was not affected by the three initial instillations and but very slightly by a fourth given 9 days later, but a fifth large instillation after another resting period of 10 days produced a well-marked attack of asthma. Guinea pig I responded, altogether, with six well-defined paroxysms of asthma, but during the last 168 days of treatment 11 large instillations were given, none of which caused asthma. Guinea pig III received 21 large and 43 small instillations; the object of the latter was, by a nonprovocative dose, to avert the symptoms induced by the large instillations.

The sensitization in guinea pig III proceeded steadily, was not averted by four small instillations on alternate days and on the ninth day after the preliminary dosage led to pronounced asthma under the stimulus of a large instillation. Guinea pig III developed but three attacks of asthma at widely separate intervals. During the last 159 days of treatment the animal received 16 small and 10 large instillations without asthma. It appeared, therefore, that in both guinea pigs I and III the asthma-mechanisms were immune to the stimulus of horse serum. It was obviously of interest to determine whether this local resistance predicated a loss of general sensibility towards the antigen.

Accordingly 17 days after the last instillation, guinea pigs I and III received an intravenous injection of 0.25 c.c. horse serum and each promptly died in typical shock. At the autopsies no macroscopic abnormality could be discerned except deep congestion of liver and kidneys in each and excess of mediastinal fat in III. There was no evidence of fatty degeneration. These results are rather surprising in view of the visceral degenerations obtained by Longcope<sup>12</sup> following repeated injections of foreign proteins. It may be surmised that the pathologic tissue change depends upon the *amount* of foreign protein introduced rather than on the reaction of anaphylaxis.

Guinea pigs II and IV were from the same immunized mother but of different litters.

Guinea pig II was extraordinarily sensitive to intranasal dosage.

Among many guinea pigs similarly tested, this was the only one that ever responded with asthma at the very first instillation of horse serum into the nose. It will be noticed that early in the series of observations even a small instillation was once able to provoke an attack of asthma.

The animal was observed through 397 days, receiving in that time 35 large and 74 small instillations of serum. It will be observed that the small instillation of serum given on alternate days has a restraining influence on the response to the larger instillations and that this property is not so marked when the small

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The black crosses represent small instillations (0.04 c.c.); the red crosses, large instillations (0.2 c.c.).

A single cross indicates no reaction; two crosses in the same ordinate indicate slight; three, moderate; and four, severe asthma.

Read from left to right.

to the larger instillations and that this property is not so marked when the small

instillations are given daily, as was done towards the end of the experiment. It is obvious, also, that although the hypersensitiveness of the respiratory apparatus became for a time allayed, it returned later in full force.

The important conclusion may be drawn from the record of this animal, strengthened by the results in other cases, that the small instillations of serum have a positively inhibiting effect over the development of anaphylaxis, but that hypersensitiveness is maintained by repeated applications of the larger dosage.

The inhibiting effect of the small instillations of serum is well illustrated in the history of guinea pig IV. This animal was observed through 397 days, receiving in that time 38 large and 20 small instillations of serum. For the first 240 days of treatment, the pigs received only large instillations of serum, to the number of twenty-four, every one of which was attended by a well-marked attack of asthma, some of the paroxysms being very severe. It will be observed that when four small instillations of serum, indicated by black crosses, were given on alternate days, the succeeding two large instillations for the first time provoked no asthmatic response, and that finally a strong degree of resistance against the large dosage was developed.

It may be objected that the inhibiting effect of the small instillations of serum over the development of the asthmatic attack is merely an illustration of "antianaphylaxis."

A substantial basis for this interpretation is afforded by the fact that, as a rule, only a 24 to 48 hours interval was allowed to elapse between the last of a series of small, preventive, instillations and the large provocative one. Skipping to a consideration of the history of guinea pig VI, we find that the known conditions of antianaphylaxis do not explain the sedative influence of the small instillations.

Guinea pig VI was observed through 382 days, during which it received 25 large and 47 small instillations. The initial treatment differed from that given the other animals, and a paroxysm of asthma was not produced until the fifth instillation on the twenty-ninth day. It should be recalled that the intranasal treatment of this animal began immediately after it had received its subcutaneous injection of serum. During the incubation period of the latter there was no respiratory reaction. That the animal was extremely hypersensitive is shown by the fact that on four occasions asthma was caused by small instillations. In this pig the groups of small instillations were, as a rule, separated from the large provocative dosages by resting intervals of ten days or more. It seems obvious from the result that there was an inhibiting effect of the small instillations which reached over the ten days' interval, whereas, as in the cases of the other animals, the larger instillations apparently progressively increased the sensibility; though the antianaphylactic effect of the larger dosage should presumably have been greater.

Again we find a somewhat less inhibiting effect produced by small instillations repeated daily than when given on alternate days.

These data of time and amount of dosage have, of course, great practical importance for those who would use them as guides to therapeutic application.

We have, finally, to consider guinea pig V which differed from the others in being born of a normal mother and in having been sensitized by relatively massive injections of horse serum into the abdomen and under the skin. In 397 days this pig received 34 large and 75 small instillations of serum.

The biologic character of the reactions closely resembles those found in the other cases. There is the same tendency of the small instillations to inhibit and of the large ones to excite anaphylaxis. The persistence of the state of hypersensitiveness is well illustrated in this animal. The very last instillation to which he was subjected produced the severest reaction of all; for many minutes there was evidently a bronchial spasm so severe that it seemed as though the animal must expire for want of breath. No convulsions marked this stage.

This tedious work has resulted in no discovery, but it seems worth recording as a purely experimental essay in an important field of anaphylaxis. Some of the essential principles illustrated by these experiments were expounded by James Paget in a clinical lecture<sup>12</sup> more than forty-six years ago. He dwells upon the immunity to dissection intoxications induced by frequent exposures and the reacquirement of sensitiveness by a sufficiently prolonged period of rest.

#### SUMMARY.

It has been shown that guinea pigs, especially after previous sensitization, may react to intranasal instillation of horse serum by the development of respiratory seizures which have the essential characters of bronchial asthma.

In some cases an apparent immunity of the respiratory apparatus may be developed while general hypersensitiveness still remains, as proved by the fatal effect of intravenous injection of serum. In most cases the hypersensitiveness of the respiratory apparatus once acquired tends eventually to return, at least through a period of thirteen months.

Small nasal instillations of serum, 0.04 c.c., not themselves usually capable of inducing asthmatic paroxysms, apparently have a distinctly inhibiting effect on the development of asthma by large instillations, 0.2 c.c., which themselves tend to maintain hypersensitiveness and override resistance.

It is an opinion supported by many additional observations, that serum is actually absorbed by the mucous membrane of the nose whenever placed in contact with it and that the immunologic reactions are internal to the surface.

The conclusion, emphasized in other papers, is here strengthened that in therapeutic prophylaxis better results may probably be expected by choosing a dosage of antigen which just fails to produce an obvious reaction than by one which entails a marked disorder.

It is a pleasure to acknowledge indebtedness to Dr. Cuthbert Powell for performing the operative work necessary in two cases.

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# CASES OF CHRONIC INFECTION APPARENTLY FOCAL IN THE GALL BLADDER\*

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## CASE I.

J. R., Hospital No. B-1182, a white man 70 years of age, was admitted to the Cincinnati General Hospital on Feb. 17, 1917, in a semiconscious state and distinctly under the influence of alcohol. On admission the pulse was 88 and of fair volume and quality. The reflexes were normal. There was no bleeding from the ears, nose, eyes, or mouth. The scalp was bruised but not broken. There were no pressure symptoms. The day after admission the patient appeared to be clearer, mentally, but could not speak distinctly. Upon the lower lip was an ulcerated area which had been the seat of a previous operation for cancer. Both eyes were contused and "black." The patient was not able to grasp or hold objects. He took food well. An x-ray plate showed nothing definite. At times the patient was delirious, and he voided involuntarily.

On Feb. 20, 1917, the pulse began to increase in rate and the patient sank rapidly and died at 10:30 P.M.

CLINICAL DIAGNOSIS.—Concussion; probable fracture of the vault; arteriosclerosis; alcoholism; carcinoma of the lip; senility.

## AUTOPSY PROTOCOL.

The body was that of a well-built, fairly well-nourished old man of apparently 60 to 65 years of age. Over the anterior surface of the body, particularly over the left anterior superior spine, in a region just beneath the left nipple and at various points over the thorax, particularly to the left, were numerous greenish bruises. There was a bruise just above the right wrist. Over the vertex of the skull was a linear lacerated wound which was closed with sutures and which did not extend to the periosteum. The pupils were equal and slightly dilated. The mouth was in an exceedingly bad condition. There were many carious snags remaining in the gums and one or two long irregular teeth. There was a hemorrhagic suffusion about both eyes. Practically the whole of the lower lip near the margin was the seat of a hyperplastic epithelial process the surface of which was ulcerated and irregular. Just at the right angle of the mouth was a scar remaining from an old operation. The skin covering the lower half of each leg was brownish, atrophic and squamous and had a board-like feel but there was no edema. Just above the left internal malleolus was an area of ulceration covered with dried crusts. Thirteen centimeters above the right internal malleolus were two crusted lesions that superficially resembled psoriasis except that the crusts were more hyperplastic. Beneath these crusts the epithelium appeared to have formed long hyperplastic papillae. Both legs above these areas of pigmentation showed numerous dilated veins.

When the calvarium was removed, it was found that the dura was very unusually adherent. The pia was everywhere edematous but there was no evidence of hemorrhage. There was an increased amount of spinal fluid and the blood vessels of the base showed patchy arteriosclerosis which was particularly prominent in the middle cerebrals. There was an increased amount of fluid in the ventricles which were quite dilated. There was no evidence of hemorrhage in the brain. There was no evidence of fracture of the skull. Rigor mortis was present; posterior lividity was brilliant. The subcutaneous fat was exceedingly well developed. The omentum formed an apron over the anterior surface of the intestines and was well supplied with fat. The appendix was *in situ* and ran up closely adherent to the posterior surface of the cecum. There was no other abdominal lesion.

When the sternum was removed, the lungs did not collapse. There was no fluid in either pleural cavity. The 5th, 6th, 7th, 8th and 9th ribs were fractured in a line cor-

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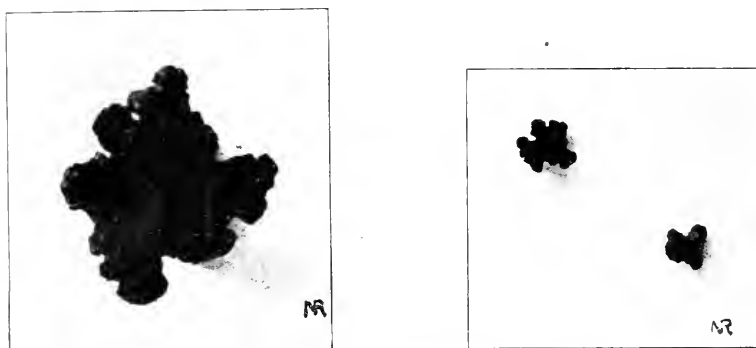


Fig. 1.—Gall stones from Case I.

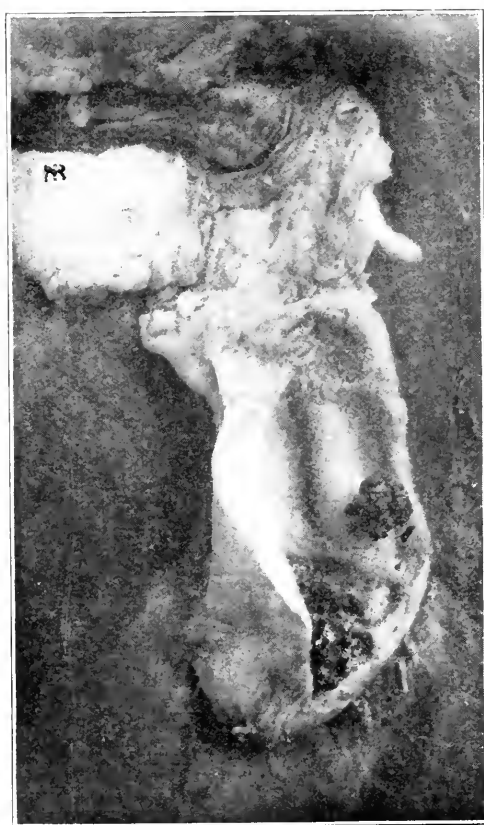


Fig. 2.—Gall bladder of Case I. Note the two areas of ulceration and also the odd-shaped gall stones.

responding to the anterior axillary line, but the pleura was not broken at any place. There were no adhesions in the left pleural cavity but there were numerous old apical and diaphragmatic adhesions in the right. There was no increase of pericardial fluid. There seemed to be no appreciable enlargement of the cervical lymph glands. The large intestine was tremendously distended with gas. There was a very large increase of perirenal fat.

The right kidney was of fair size, the capsule removed with ease. The whole organ was pale and evidently edematous. The cortex was narrowed. The line of de-

marcation between cortex and medulla was distinct. The glomeruli were occasionally visible as congested points. There was evident sclerosis of the pyramids. The friability of the whole organ was decreased. There were very numerous petechial hemorrhages in the pelvis. The left kidney resembled in every respect the right. There were a few small retention cysts in each kidney. The spleen was small and flabby, the capsule smooth, and the pulp rather pale, but otherwise there was nothing unusual.

The liver was small, the surface was everywhere pale and smooth. On section the parenchyma had a diffuse cloudy appearance and nothing more. The friability of the organ was decreased. The gall bladder was small and contracted and contained a number of calculi; pressure upon the gall bladder caused the appearance of a thinnish mucopurulent material. The bile ducts themselves were patent. The calculi in the gall bladder were typical jack-stones in appearance and were quite firm. The gall bladder itself showed a thickened fibroid wall and the distal half was the seat of a complete circular ulceration. The base of the ulceration was yellowish, soft and mucoid. The urinary bladder contained a large amount of somewhat cloudy amber fluid. The mucous membrane, however, was healthy. The prostate was moderately enlarged.

The heart was rather large and was dilated on the right. The tricuspid and pulmonary orifices seemed to be healthy. There was no essential lesion of the mitral or aortic leaflets. In the aorta just to the right of the innominate was a large atheromatous ulcer which extended into the innominate. Throughout the rest of the aorta there were scattered areas of subintimal fatty degeneration with quite numerous atheromatous abscesses and ulcers. These were particularly well marked in the eight centimeters above the bifurcation and in this area the ulcers were all pigmented.

The right lung was diffusely edematous and the posterior half of the upper lobe seemed to be partially consolidated. Over the pleura was a very fine fibrinous exudate. There was some slight exudate also over the lower lobe which was less obviously consolidated. Otherwise the pleura was smooth. On section it appeared that the posterior half of the upper lobe was almost completely consolidated and in a state of red hepatization with some small areas of gray. The lower lobe showed a more diffuse red hepatization with more edema. The bronchi were all intensely congested and contained a cloudy blood-stained serous fluid. The apex was scarred. The left apex was also scarred and hyperplastic. The upper left lobe was completely crepitant and the lower lobe was not completely crepitant though it contained air throughout. This lobe was distinctly edematous and evidently in a stage of inflammatory edema. The pleura showed some fine fibrinous exudate.

The stomach and intestines showed nothing unusual. The pancreas was apparently healthy.

**ANATOMIC DIAGNOSIS.**—Chronic suppurative cholecystitis; cholelithiasis; acute lobar pneumonia; acute fibrinous pleuritis; arteriosclerotic kidney; aortic atherosclerosis; myocardial fibrosis; acute nephritis; edema of the brain and meninges; multiple superficial contusions; fracture of the 5th, 6th, 7th, 8th, and 9th ribs; epithelioma of the lip.

#### REMARKS.

In this case no cause is recorded which may be related to the gall bladder condition. The protocol is presented because, so it seems, the evidence contained in it points to the possibility that given a focal infection and a generally decreased resistance—a lowering of vitality,—the chances of a serious acute infection by the hematogenous route are multiplied. What seems to have happened in this case is that an old alcoholic man was "beaten up" and so badly damaged that opportunity was given for organisms whose home was in the gall bladder, to attack the lungs and set up an acute lobar pneumonia, of a somewhat unusual type.

#### CASE II.

H. H., Hospital No. B-881, a white woman 55 years of age, was admitted to the Cincinnati General Hospital on February 6, 1917. Her complaint was that she had fainted in a shop where she was making a purchase.

**FAMILY HISTORY.**—Her mother died at the age of 58 in a sanatorium to which she had been sent for melancholia.

**PERSONAL HISTORY.**—She was married in 1882, and had one child who died of diphtheria at the age of 12. She had had no other pregnancies. The husband died at the age of 55, of pneumonia. She gave no history of diseases of childhood. At the age of 15 she had typhoid, and later at different times had rather mild attacks of rheumatism. About five years before admission she had severe continued pain in the little toe of the right foot, and the toe was amputated. Since that time the sensation of pain and also twitching has persisted at the site of the operation and this caused insomnia and nervousness. On the morning of the day when she fainted she had gone to the bank, and while there had been faint and dizzy. Later she went to the shop and fainted. The attack lasted but a few seconds. There was no history of trauma. For four days before admission she had a constant occipital headache, and for three days she had shooting pains in the right frontal region.

**PRESENT STATE.**—The patient is a small, fairly well-nourished, white woman. *Mental State.*—Attention is fair, but she tends to ramble in her talk. Memory is fair for past events; dates are very indefinite. Patient seems to be slightly dulled. Questions must be repeated, answers are often irrelevant. *Special Senses.*—Slight diminution for counting fingers at a distance of two feet; more with right eye. Patient seems slightly deafened; taste and smell subjectively normal. *Cranial Nerves.*—Pupils equal, small, fixed to light and accommodation. No consensual or sympathetic reaction. Sensation over the face seems normal; ocular movements normal. Face appears symmetrical. Facial movements normal except in showing her teeth the patient draws up right side of the mouth more. Articulation, phonation and deglutition normal. Shoulders shrugged equally; pulse normal. Tongue protruded in midline, no tremor. *Sensory System.*—Scattered analgesia over legs, arms, abdomen and chest; joint sense normal. Patient complains of pain in left shoulder joint. *Motor System.*—Patient says the left arm is weak and will not attempt to grasp with it. No unusual wasting; strength in legs normal. No contractions, tremors, or spasms. *Reflexes.*—No knee jerks; abdominals diminished if not absent. Clonus and Babinski negative.

*February 7.*—The upper third of the left arm is discolored a purplish-red, is swollen, hot and tender. On palpating line of tenderness is felt near the head of the humerus with definite crepitation. Sent for x-ray.

*February 18.*—Transferred to surgical service. Taken to x-ray department and her arm put up in a case encircling her chest, which held the arm tight to the side. X-ray showed a fracture of head of left humerus with some displacement of fragments.

*February 22.*—The patient became very weak, her heart rapid, and pulse feeble and compressible. She died at 10:25 P.M.

**CLINICAL DIAGNOSIS.**—Tabes dorsalis (paresis?); fracture of the neck of the left humerus.

#### AUTOPSY PROTOCOL.

The body was that of a fairly well built, moderately nourished old woman of apparently 60 to 65 years of age. There was a slight edema of the ankles. Rigor mortis was present; posterior lividity was present but not brilliant. Over the right buttock was a small clean bed sore. Over the left buttock was an area of excoriation about 4 or 5 cm. in diameter. The upper left arm, from a point just about the neck of the humerus to a point half way between the elbow and wrist, was almost diffusely discolored, purplish and greenish, and was edematous. Rotation of the arm showed crepitus at about the neck of the humerus. The left wrist showed a distinct silverfork deformity and the left hand was atrophic. There was a large bruised area with edema over the upper left part of the thorax anteriorly between the clavicle and breast. External to this there were some purplish ecchymoses and low in the axilla was another patch of subcutaneous suffusion.

When the spinal cord was removed it was found that there was a large increase of clear spinal fluid so that the dura was much distended. There was an increased amount of fluid beneath the dura of the brain. Also the pia was decidedly edematous, particularly over the vertex and the convolutions, particularly the frontal, were somewhat atrophic. The sulci over the right vertex were rather widened and deepened but there was no

evidence of inflammatory change. The pia was slightly clouded. The blood vessels of the base were tremendously arteriosclerotic, to such an extent that in removing the brain the basilar artery fractured across and tore readily. The cerebellar branches, but particularly the middle and posterior cerebals, were exceedingly sclerotic and tortuous.

The peripheral lymph glands were not appreciably enlarged. The pupils were equally contracted. The lower front teeth were all that remained and they were in a fairly good condition. There was no pyorrhea but there was a fairly large amount of tartar about all of the teeth and the gums were pigmented. The subcutaneous fat was very well developed; the muscles were pale. The mammary glands were evidently healthy. The lower margin of the liver lay 10 cm. below the ensiform process and  $4\frac{1}{2}$  cm. above the costal margin in the right mammillary line. The appendix was *in situ* and evidently healthy.

When the sternum was removed, the lungs did not collapse and in the mediastinal fat there was a small amount of hemorrhage that reached from just beneath the manubrium to 10 cm. below it. The left pleural cavity was almost completely obliterated with old fibrous adhesions. In the right pleural cavity there were a few old apical and lateral adhesions. The bronchial lymph glands were almost completely calcified. There was no increase of pericardial fluid.

Dissection of the left shoulder showed that there was a simple comminuted fracture of the neck of the humerus.

There were some old adhesions between the gall bladder, stomach and hepatic flexure of the colon. After removal of the duodenum, pancreas and stomach, there appeared, just below the line of the left renal vessels, a pigmented area, quite firm in consistence, the tissue of which was quite friable. When it was torn the finger could be introduced apparently into the left kidney. There was no distinct odor connected with it and the fluid which ran from it had a dark, almost greenish, cloudy urinous appearance. It had no connection with the renal vessels. Through the opening made into the urine-containing sac, the finger could be extended easily downward along the ureter which was quite evidently slightly dilated. In the region of the right broad ligament was what appeared to be a lipoma almost as large as one's fist. The urinary bladder contained a large amount of pale, somewhat clouded urine. It was very markedly trabeculated; there was no evidence, however, of any inflammatory process. The left ureter was apparently healthy. There was nothing abnormal to be seen in the course of the right ureter. On section of the left kidney it was found that the pelvis was very large, dilated, and marked with very numerous petechial hemorrhages. The dilatation extended into the calices and the substance of the kidney had been compressed. In spite of a rather well marked edema of the parenchyma, the cortex was exceedingly narrow, averaging about 3 mm., was pale, the line of demarcation between cortex and medulla was distinct but not brilliant, but in neither cortex nor parenchyma was there any evidence of macroscopic inflammatory process. The capsule removed with difficulty leaving a finely granular gray surface. The evidences were that the pelvic inflammatory condition had extended into the pelvic fat and thence into the perirenal tissues. The right kidney showed very much the same condition in a less advanced stage, however, and with no perirenal invasion. The tubes and ovaries were apparently healthy. There was a slight erosion about the mouth of the cervix uteri and the uterus as a whole was very atrophic and what had seemed to be the body of the uterus was merely a single mural fibroma.

The liver was small and brown. The capsule was for the most part smooth, and there was an accessory groove parallel to that of the suspensory ligament and just at the lower part of this was an area of superficial scarring somewhat stellate in shape, but not extending deeply into the tissue. The organ was very friable on section and quite brown. The gall bladder contained two gall stones buried in a thick greenish-yellow pus. The walls of the gall bladder were thickened, fibroid, and in part ulcerated. The right lung crepitated throughout. The apex was scarred and there were no adhesions between the lobes. There was a slight amount of hypostatic congestion. On section nothing remarkable was evident except a moderate edema. The left lung was also crepitant throughout. Its surface was tagged with old adhesions and the lobes were bound firmly together. The apex was scarred and at one place in the lower lobe was a fibroid patch containing lime salts. Section showed nothing except a moderate hypostatic congestion. The heart was of fair size. The coronaries were somewhat sclerotic and quite tortuous. The pulmonary and tricuspid valves were evidently healthy. The mitral valves were thickened at the edges but there was nothing else noticeable. The aortic valves also were reasonably healthy.

The aorta was thickly patched with areas of fatty degeneration and some atheroma. This atheromatous process was still more marked in the lower abdominal segment where it was represented in the narrowed vessel by pigmented ulcers, discrete and confluent. In the neighborhood of the origin of the renals and in a few inches just above this there were a few hyperplastic subintimal fibrous patches. The spleen was small. The capsule was smooth. The pulp was gray, the Malpighian bodies were not visible. The stomach showed nothing unusual. The duodenum contained bile-stained fecal material. The pancreas seemed to be somewhat edematous and to be the seat of a moderate fibrosis with some fatty infiltration. The rectum was the seat of a catarrhal condition so marked that all the layers of the gut were exceedingly edematous. The mucous membrane was particularly thickened and the tops of the rugæ were distinctly hemorrhagic; above the rectum the gut was still congested, particularly so in the cecum. The small intestines seemed to be relatively healthy.

**ANATOMIC DIAGNOSIS.**—Comminuted fracture of the neck of the left humerus; chronic suppurative cholecystitis; cholelithiasis; acute pyelitis; chronic diffuse nephritis (interstitial type); acute perinephritis; obsolescent pulmonary tuberculosis; acute hemorrhagic proctitis; aortic atherosclerosis; cerebral arteriosclerosis; cerebrospinal edema; atrophy of the cerebral convolutions; bilateral hydronephrosis; tabes dorsalis (?).

#### REMARKS.

In this case, as in the former one, a focus of infection was present in the gall bladder. In this, however, there was a possible etiologic factor in an early attack of typhoid. It is possible that some other cause might have been elicited for the cholelithiasis had the patient been in a better mental condition. Be that as it may, the patient, arterially diseased and mentally damaged, suffered a very severe trauma, and following that died somewhat unaccountably. At autopsy nephritis and pyelitis were present which is interpreted as evidence of a renal infection secondary to the cholecystitis, and induced by the trauma and its consequent effect upon the general resistance of the patient.

Sections through the cortex of the brain showed marked disorganization of the cortical striations with great morphologic changes in the individual cells, chromatolysis, eccentricity of the nucleus and satellitosis. Noguchi preparations were doubtful and definite demonstration of spirochetes could not be asserted. Kulchitsky's preparations of the spinal cord, from the cervical, dorsal and lumbar regions showed a moderate degeneration of the columns of Goll in the last mentioned level.—[*W. E. K.*].

#### SUMMARY.\*

One of the interesting features connected with these cases is that they came to autopsy almost at the same time. Another feature is that one might suspect that the lobar pneumonia in Case I was the result of alcoholism and exposure, rather than of metastatic infection. Perhaps this was really so, but in the second case there was no exposure or alcoholism and the evidences point to metastasis facilitated by trauma as the essential factor. Taken together these cases suggest the definite relation existing between chronic foci of infection and a terminal acute metastatic infection. In neither case was there any history of gall bladder disease.

\*For a general discussion of focal infection, see Billings: *Focal Infection*, New York, 1916.

## ACUTE DEATH FROM CHLORINE POISONING\*

BY OSKAR KLOTZ, PITTSBURGH, PA.

THE study of the effect of chlorine gas poisoning is one that has been introduced by the present war. Prior to 1915 there are but two or three references in literature concerning the harmful action of chlorine upon health. To have undertaken an extensive study of chlorine poisoning, would have been looked upon as an academic occupation of one who had difficulty in finding a problem of active interest to human welfare. Suddenly, however, the medical officers, serving on the battlefields, were confronted with the direful effects of chlorine and other gas poisonings for which their studies in therapy had given them no adequate remedy. In the absence of knowledge of the manner in which these irritating gases produce their effects, the resources of chemists and physicists were called upon, to offer a means of prevention. Our hopes along this line of endeavor have been greatly raised by the introduction of adequate masks. Nevertheless, before these became available, much harm has been done both through the gruesome method of killing men, as well as the permanent injury inflicted upon those receiving a lesser concentration of gas. Chlorine appears to have been used most extensively, but bromine, phosgene and other gases have also been used.

The horror of the first gas poisonings led to a number of investigations by British authors. These studies have been of an experimental kind and considerable information has been gained as to the manner in which chlorine attacks the tissues. Schaefer has exposed animals to chlorine gas varying in concentration from 1 to 20 per cent. Such concentrations are unusually high and it is improbable that any of the soldiers at the front have ever found themselves in an atmosphere containing this amount of chlorine. It is more likely that the human gasings are accomplished in a concentration of 1:1000 to 1:10,000. However, each gas raid by the Germans must vary in the actual amount and concentration of gas which reaches the allied forces. The efficiency of gassing by chlorine rests to a large extent upon the climatic conditions, there being necessary a favorable breeze sufficient to waft the liberated chlorine towards the opposing trenches, but with the air currents inefficient to stir up the heavy gas from its low lying position over the terrain to cause excessive diffusion. Furthermore, the concentration of the gas will be materially altered if on the morning of its use, the ground be damp and the air contain much fog. The great solubility of chlorine gas in water permits its rapid removal from the air.

Schaefer's important observations on chlorine poisoning may be summarized as follows. The immediate effect of chlorine gas is to irritate the mucous membranes with which it comes in contact and particularly the bronchioles. The bronchial tubes did not appear to alter the size of their lumina by muscular contraction and Schaefer did not believe that the dyspnea was referable to the contraction of the air tubes. It was observed that the main effect of the inhalation of gas was upon the lung structure wherein the capillaries responded in an

\*From the Pathologic Laboratories, University of Pittsburgh.  
Submitted to the National Research Council by the author.

unusual dilation followed by edema of the alveolar walls and the air sacs. With this pulmonary congestion, there was a marked drop in the systemic blood pressure, which, in the absence of a primary cardiac failure, indicated some resistance to the blood flow from right to left heart. Schaefer refers to this occurrence in the terms of an "obstruction in the pulmonary vessels rendering it impossible for the blood to pass freely to the left auricle and ventricle." He was unable to indicate the exact manner in which this pulmonary obstruction was brought about.

Shortly following this, Leonard Hill also studied the problem of gas poisoning, paying particular attention to the effects of chlorine. His viewpoint differs somewhat from that of Schaefer. Broadly, his attitude is that chlorine is a definite irritant, acting not unlike a burn. In other words, the application of chlorine to tissues will simulate in its reaction other irritants which induce various grades of inflammation. The exact quality of the reaction will be determined by the amount and concentration of the irritant substance. Thus, at times, in high concentration, chlorine will destroy the cells with which it comes in contact. The bronchi and bronchioles may be denuded of their epithelium, and the irritation of the gas upon the deeper structure of the bronchioles will lead to muscular spasm unless the gas has destroyed these elements. Hill thoroughly agrees with Schaefer that, since chlorine is such an active chemical agent which can find so many substances with which it can unite in the blood, it will not be carried in the free state to distant parts. Thus it is hardly probable that chlorine, as such, damages distant tissues, but only accomplishes serious organic lesions, such as described by Broadbent in the kidney, through indirect means. Hill, furthermore, observed that at the beginning of the experiment, when chlorine was first given the animal, the expansion of the chest was diminished, which he believes was due to the contraction of the bronchial tubes. Congestion and edema of the lungs followed, appearing first in patches and then spreading. The blood became venous and the output through the lungs was lessened. If the edema of the lungs was forcibly removed by pressure, the condition of the animal was greatly improved.

The problem concerning the deleterious effects of chlorine bears a direct relation to respiration and the pulmonary circulation. For the present, we will not concern ourselves about the secondary manifestations which may arise in a vicious circle. It is clear that systemic poisoning does not occur, and the distant effects, whatever they be, are in large part the result of the intrathoracic pathology.

Hill describes a typical human case of gassing as one that is "cold with a subnormal temperature, conscious but restless, with pulse slow and full (except in the collapsed cases). The face is cyanosed, intensely so in many cases, and the expression strained and anxious. The posture varies. In some cases the patient sits propped up, with head thrown back, gasping for breath; in others, he lies on his side, with his head over the edge of the stretcher in an attempt to aid expectoration. The respirations are jerky and hurried, often numbering 40 a minute, and are associated with a choking cough, accompanied by a varying amount of frothy expectoration. With each inspiration the chest is expanded to its fullest, all the auxiliary muscles being brought into play just as in an asthmatical paroxysm. This is the first, or asphyxial, stage, which, if the pa-



tient survives, gradually passes off after some thirty-six hours. After the first stage the patient falls into a sleep, and awakes feeling much better. But after a few hours of comparative quiet, symptoms of bronchitis begin to manifest themselves. In the majority of cases these are not severe, because, no doubt, nearly all the severe cases die in the first stage. In the cases that are kept alive with difficulty there is a short quiescent stage followed by intense bronchitis. The frothing gives place to greenish mucopurulent expectoration, consciousness to delirium, the temperature rises from subnormal up to  $104^{\circ}$  F., the pulse becomes of small volume, with its rate increased perhaps to 160, the respirations are less choking but more shallow, and number up to 70 a minute before death."

This description of gas poisoning in man is very similar to that noted in animals. When guinea pigs are exposed to 1:1000 chlorine gas, evidence of irritation is seen in the watering of the eyes and in the attempts on the part of the animal to seek a position away from the fumes. For a time, the animal appears to be holding his breath. Soon, however, he is forced to inspire, causing him to cough and sneeze. With this, there is a watering of the nose, and the animal paws his face as if to brush away the irritant. The respirations now become more rapid but jerky; there appears to be some difficulty in breathing and the thorax is held quite rigid while the costal borders tend to flare and shallow respirations are carried on mainly by the diaphragm. The animal is distinctly distressed, and gives intermittent gasps. If at this stage the animal is removed from the chlorine mixture, the gasping continues for some time, even hours, as he is recovering. If the animal has been overexposed the gasping continues after he is in the free air, until death. When allowed to remain in the gas mixture, the respirations shortly before death become hurried and then suddenly cease. During this time the heart beats regularly and continues for some time after the cessation of respiration.

Acute chlorine gas poisoning may be induced in small animals, mice, guinea pigs, and rabbits in concentrations of from 1:10,000 to the stronger mixtures. For careful observations it is well not to use mixtures of a greater concentration than 1 per cent. The main differences that we have noted in the various strong concentrations of chlorine gas have been in the rapidity of the onset of the symptoms and the time of death. In the very acute cases, death occurs in 3 minutes; with the less concentrated mixture, rabbits, guinea pigs, and mice will survive for half an hour or longer.

Some observations were made upon the blood of gassed animals. As we have stated above, chlorine is not absorbed and distantly distributed in the free state by the blood. In a previous study by Miller, it was shown that a definite lymphocytosis was present in cases of chronic chlorine poisoning. These chronic cases were soldiers who had survived the gassing for several weeks or months. In the acute cases, as was observed in one of his cases no lymphocytosis was present. He believes that an increase in lymphocytes was due not to the direct effect of the chlorine gas but was the result of chronic inflammatory changes in the lungs. When chlorine gas, even in very low dilution, is permitted to come in contact with blood, its active reducing qualities are quickly noted. The blood in contact with the chlorine becomes black and gummy and on long exposure is decolorized. By passing small quantities of chlorine through a blood solution, the coloring matter is entirely destroyed and the iron is liberated in the watery

solution. Hake demonstrated this free iron by Prussian blue test. He suggested that this marked effect of chlorine on the blood, might have some relation to its lethal effect. In none of our animals have we been able to demonstrate such profound blood changes referable to the chemical effects of chlorine.

On the other hand, an interesting observation was made upon the cellular content of the blood of the treated animals. When mice were exposed to the lethal concentration of the chlorine gas, it was found that immediately after death, the congested areas of the lung contained but small quantities of fluid blood. The congested areas stood out prominently and did not shrink or collapse. The tissues were edematous and there was frothy fluid in the bronchial tubes but the blood within the congested portions of the lung substance appeared coagulated. On several occasions we were surprised to find that this coagulation proceeded into some of the larger vessels. The right heart, both auricle and ventricle, was dilated and continued beating after cessation of the respirations; the left heart was in firm systole. The systemic circulation had ceased and respiration was no longer maintained, owing to cerebral anemia. It appeared as Schaefer intimated, that there was some obstruction in the pulmonary circulation. It was difficult at first to account for such an obstruction when in truth the vascular channels in the lung were in a state of congestion. Here, however, there was evidence that in these cases of acute poisoning, the stage of congestion was only an early manifestation of the irritating effects of the gas and that this congestion was succeeded by a stage of intravascular coagulation. The pulmonary tissue responded to the irritant, with a type of inflammatory exudate in which large quantities of serum escape from the dilated capillaries and permeate the alveolar walls and air sacs. The acute deaths in animals were always accompanied by a remarkable pulmonary edema. The lungs were waterlogged and the alveoli filled with a thin frothy fluid.

Even with the intense congestion of the lung we have never observed hemorrhage sufficient to tinge the edematous fluid. The remarkable rapidity and extent with which this edema develops, suggested the possibility that the abstraction of water produced marked changes in the blood within the pulmonary capillaries. The possibility that this abstraction of water and consequent thickening of the blood increased the viscosity and led to the excessive resistance of the blood flow in the lungs is in agreement with the findings of Schaefer and the demonstration of the right heart embarrassment. It, furthermore, occurred to us that the abstraction of these relatively large quantities of fluid from an engorged system of vessels would not only increase the density of the plasma but would also bring about a change in the numerical cellular content of the blood. That this was the case was demonstrated in blood counts made from the pulmonary vein of the gassed animals (mice and guinea pigs), such counts showing an increase of from two to five million red cells over the normal count. It was found, therefore, that the obstruction in the pulmonary circulation referred to by Schaefer was in part at least due to the increased viscosity of the blood.

There is, however, another factor of much graver importance affecting the pulmonary circulation. As we stated above, the congested areas of the lungs were found to contain less fluid blood than would be expected from their appearance. From the character of the dry congested tissues of the lung it was evident that the blood had coagulated within the dilated capillaries. This state

we found only in the severely and fatally gassed animals. As we will mention later, microscopic evidence of such coagulation was obtained. This unusually rapid coagulation may be the result of the intense edema whereby the blood constituents within the vascular channels are greatly altered and concentrated, permitting of rapid spontaneous solidification. On the other hand, chlorine gas, when in contact with fluid blood, tends to coagulate it almost instantly. We have found that human blood placed in an atmosphere of 1:1000 of chlorine coagulates in about fifteen seconds; stronger concentrations reduce this time. It is probable that within the lung where, through congestion and edema a slowing of the capillary circulation has already taken place, and where because of the great abstraction of water from the local tissues, the blood is in the threshold state for coagulation, the local and direct effect of chlorine upon the capillaries and blood materially hastens coagulation.

In as much as in our experimental animals the respirations ceased before the stoppage of the right heart, and as Schaefer has shown that there is a distinct drop in the arterial blood pressure, it would appear that the acute deaths of the experimental animals were the direct result of obstruction of the pulmonary circulation and that the presence of the pulmonary edema was a factor in this regard.

But, however, all of the acute deaths taking place from a few minutes to an hour after exposure observed in human cases are not as intense as those described. Under those circumstances where the individual continues to live for an hour or two, the fatal result is more particularly associated with the edema and consequent asphyxia than to a direct embarrassment of pulmonary circulation, although undoubtedly the increased viscosity of the blood associated with pulmonary edema also plays a part.

Our microscopic analyses substantiate the above findings and indicate that the important changes take place in the lungs while tissues elsewhere in the body are not directly affected by the gas. We have repeatedly observed the absence of congestion in the abdominal organs, though in animals surviving some hours after gassing, the liver showed a mild grade of engorgement. This was in consequence of the inability of the right heart to carry on an adequate circulation through the lungs.

The reaction (microscopic) in the lung varied according to the length of time of exposure to chlorine and with the concentration of the gas used in the experiments. The mildest reaction consisted of congestion, effecting particularly the capillaries of the alveolar walls and also the larger blood vessels, both arteries and veins. When the congestion had continued for longer periods of time, an edema made its appearance within the air sacs. This edematous fluid is of a very watery nature and of low albuminous content. The alveoli are flooded with this fluid and when it persists, a gradual shrinking in the size of the alveoli takes place. Concurrently with the appearance of this edema, scattered portions of the lung become emphysematous. These emphysematous alveoli become widely stretched, and are devoid of fluid. In this stage, congestion, edema, and some emphysema are the outstanding pulmonary changes. It is to be remarked that although the congestion is very intense and has an appearance as if the small alveolar capillaries would rupture, it is but rare to

observe the presence of red blood cells within the air sacs. On the other hand an escape of red blood cells may take place into the alveolar wall itself.

A direct influence of the action of the gas upon the cells lining the bronchi and air sacs, was not frequently observed. In only a few instances, was there evidence of desquamation of a few cells of the bronchial epithelium or a change in the morphology or staining qualities of the alveolar lining cells. True coagulation necrosis was not observed and there was nothing to indicate that the contact of the gas on the mucous membranes was of particular importance in bringing about a serious pulmonary condition.

An important observation in the acute deaths, particularly in mice, was the finding of patches of diffuse coagulation of blood in the pulmonary capillaries. In these areas we have observed within the capillaries and larger vessels the presence of a diffuse meshwork like altered fibrin. Wide stretches of channels were found in which an irregular meshwork of threads stained diffusely blue with hematoxylin. In these thrombi relatively few red blood cells were found. Similar coagula with a varying number of erythrocytes were seen in the arterioles and venules. This process of thrombosis was not uniformly distributed through lung.

In those animals receiving smaller quantities of gas or in which the gas concentration was less, the fatal results were variously delayed, with this delay in time of death a considerable difference was noted in the pathologic histology of the lung. The congestion was found persisting but of a different grade of intensity although sometimes the congestion appeared equally marked as that seen in more acute experiments. The edema also persisted in different degrees, but more commonly there was a tendency for the disappearance of the watery fluid contained within the alveoli. It was not uncommon, however, that a new type of edema made its appearance. This occurred as a tissue edema, surrounding blood vessels, and to some extent around the bronchi. The perivascular lymph spaces were widely dilated, developing a loose structure around these tubes. This late edema was accompanied by a more or less inflammatory reaction in which lymphocytes and occasionally leucocytes and plasma cells were found in the fluid of the tissue spaces. Inflammatory reactions were not alone present in the perivascular tissue, but were also present in the alveolar walls, following the congested capillaries. Here again the lymphocyte was the most common cell. Leucocytes were not abundant. Endothelial cells were occasionally found within the alveoli. The extent to which the cellular exudate made its appearance in the pulmonary tissues appeared to vary, not only with the manner of exposure to chlorine, but also with the individual susceptibility of the animal. In some animals, the perivascular ring of edema became intensely packed with lymphocytes, so that a crown of cells surrounded the medium-sized vessels. times this was seen around the bronchi and occasionally the intervening air sacs contained a similar exudate.

To sum up, the acute reaction found in the lungs of animals exposed to chlorine consisted mainly in an intense congestion with edema and capillary thrombosis. Animals surviving a longer period showed a pulmonary infiltration of inflammatory cells mainly lymphocytes. The acute capillary thrombosis appears of importance in the acute deaths when the concentration of the gas is great. The intense pulmonary edema obtained by abstracting a thin serum from

the pulmonary blood vessels causes an alteration in the quality of the blood and an increase in its viscosity. This dense blood tends to clot spontaneously and is also influenced in this more rapid clotting by the presence of chlorine within the lung. These two factors, increased viscosity and capillary thrombosis, impede the ready flow of blood through the lung and lead to a fall in the arterial blood pressure. Thus, acute chlorine gassing differs materially from other forms of asphyxia.

Further studies are in progress with particular attention to the subacute and chronic types of death. I am indebted to L. E. Ramsey for practical assistance in this work.

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### BLOOD PICTURE FOLLOWING EXPERIMENTAL SPLENECTOMY\*

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IN most instances the recent reports in the literature on the blood changes in man and animals following splenectomy have shown a decrease in the erythrocytes and hemoglobin and an increase in the leucocytes. These changes have shown considerable variation both as to extent and the length of time which they last.

As early as 1878 Picard and Malassez<sup>1</sup> made observations upon the blood of splenectomized dogs. Their results have shown a diminution in the quantity of hemoglobin but no change in the number of the erythrocytes. Paton<sup>2</sup> and his co-workers concluded that removal of the spleens of dogs, cats, and rabbits had no influence upon the number of blood corpuscles. The experiments of Zesas<sup>3</sup> showed an increase in the white and a decrease in the red elements, the changes lasting until other organs assumed the functions of the spleen. Winogradow,<sup>4</sup> writing in 1882, states that there is no notable increase in the white corpuscles in splenectomized dogs. Blumreich and Jacoby,<sup>5</sup> of Berlin, found a leucocytosis in almost all instances following removal of the spleen in guinea pigs. The results of Nicolas and Dumoulin<sup>6</sup> following experimental splenectomy have shown an increase in the number of the white cells which return to normal after several months, an immediate diminution of lymphocytes followed by a passing increase with later a marked fall, very little change in the polymorphonuclears and a marked eosinophilia in only one dog. Among more recent workers Noguchi<sup>7</sup> reports a case of extirpated spleen with a large abdominal lipoma, in which, after ten months, there was a diminution in polymorphonuclears and an increase in the eosinophiles. Kreuter's<sup>8</sup> case of splenic rup-

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ture in a boy of seventeen showed a marked anemia and an enormous hyperleucocytosis eighteen days after splenectomy. At the end of six weeks the blood picture was again normal. Musser and Krumbhaar,<sup>9</sup> in their experiments upon dogs, conclude that, after splenectomy, anemia usually develops quickly and reaches its height in from three to six weeks, approaches normal after three or four months and returns to normal in five to ten months. There is a marked leucocytosis which is highest twenty-four hours after operation and remains to a slight degree for several months. They found that some variation from the above sometimes occurred but the anemia was inevitable. Lymphocytosis was not found in their series.

In a series of twelve rabbits we have made observations on the blood both before and after splenectomy. In the first four, the erythrocyte and platelet counts, hemoglobin estimation and coagulation time were made (Table I). In

TABLE I.

A TYPICAL TABLE, SHOWING RED CELL COUNTS, HEMOGLOBIN ESTIMATIONS, PLATELET COUNTS, AND COAGULATION TIME BEFORE AND AFTER SPLENECTOMY.

Days before Splenectomy	Erythrocyte Count	Hemoglobin	Platelet Count	Coagulation Time
14	5,680,000	80	1,032,000	6 min.
DAYS AFTER SPLENECTOMY				
6	4,220,000	75	1,384,000	6 "
12	4,500,000	85	1,664,000	5½ "
18	4,120,000	75	1,488,000	5½ "
25	4,740,000	75	1,196,000	5½ "
31	4,330,000	75	1,364,000	7½ "
60	5,500,000	85		6½ "
90	5,992,000	85		

every instance an anemia was produced which appeared on or before the sixth day after operation. The change in hemoglobin corresponded very closely with the change in the erythrocytes. In rabbit No. 1 there was still an anemia on the sixtieth day. This rabbit died a few days later of some intestinal disease which may have accounted for its prolonged anemia past the sixtieth day. The red cells and hemoglobin of rabbits Nos. 2 and 3 reached normal by the sixtieth day. The erythrocytes and hemoglobin of the fourth rabbit were normal on the seventy-eighth day. When technical errors are taken into consideration, no definite or constant change was found in the number of platelets or coagulation time before and after the spleen was removed. In the remaining eight rabbits leucocyte and differential counts were made. In half of the number the average leucocyte count was greater after splenectomy and in the other half the count was greater before splenectomy (Tables II and III). Counts made within the first six days after operation showed an increase in four animals and a decrease in the other four when compared to the average counts before splenectomy. In the differential counts the polymorphonuclear neutrophils showed very little change. There was a slight average increase in five, in four of which there was a corresponding average increase in the total white cell count. In six of the eight rabbits the average lymphocyte count was less after splenectomy. In every instance there was an average increase in the number of the large mononuclear elements. The transitional forms showed an average increase in four of the

TABLE II.

A TYPICAL TABLE, SHOWING LEUCOCYTE COUNTS BEFORE AND AFTER SPLENECTOMY.

Days before Splenectomy	Leucocyte Count	Poly-nuclear Neutrophiles	Lymphocytes	Large Mononuclears	Transitionals	Eosinophiles	Basophiles	Unidentified
17	4,560	35	56.5	3.5	3.5	1	5	1.5
14	5,720	50	32	11	2	1	3	1
2	4,880	43.5	40	6.5	5.5	0	4	0.5
1	5,720	48	35.5	9	5.5	0.5	1	0.5
Average before Splenectomy	5,220	44.1	41	7.5	4.1	0.6	3.2	.8
Days after Splenectomy								
1	9,860	87.5	7	1.5	3.5	0.5	0	0
2	8,040	69	7	12	8	0	4	0
4	6,200	33	50	14.5	1	0	1.5	0
6	6,100	38.5	38	14	6	0	3	0.5
10	3,520	70	17.5	5.5	3	0	4	0
18	3,200	62	17	8	7	0.5	5	0.5
25	3,800	56.5	22	13	7.5	0.5	0.5	0
32	7,760	38.5	33	18.5	6	0	4	0
60	6,240	69	14	14	2.5	1	3	0.5
82	6,720	46	35	14.5	2.5	1	1	0
Average after Splenectomy	6,144	57	24	12.7	4.7	.3	2.6	0

TABLE III.

SUMMARY OF LEUCOCYTE AND DIFFERENTIAL COUNTS BEFORE AND AFTER SPLENECTOMY.

No. of Rabbit	Average counts before and after Splenectomy	Leucocyte Count	Poly-nuclear Neutrophiles	Lymphocytes	Large Mononuclears	Transitionals	Eosinophiles	Basophiles	Unidentified
5	Before After	9,200 11,571	55.2 56.2	31.5 25.5	2.7 10.9	3. .6	0.7 .3	2.2 5.5	0 .2
6	Before After	17,025 11,873	47.7 41	21.7 33.5	10.2 12.6	2.2 2.3	1.5 1	16.7 9.3	.2 0
7	Before After	7,866 6,490	52.3 61.6	25 19.7	6.1 8.6	2.8 5.3	0 0	12.1 3.8	1.1 0
8	Before After	5,220 6,144	44.1 57	41 24	7.5 12.7	4.1 4.7	.6 .3	3.2 2.6	.8 0
9	Before After	7,220 8,557	55 60.4	27.7 29.9	3.7 6.2	5.7 1.9	3.5 .6	2.2 .8	0 0
10	Before After	9,900 10,507	41.5 44.5	36 29.5	6. 14.1	2.7 2.7	.5 .4	3.2 8.4	0 .1
11	Before After	11,000 8,871	44.5 44.2	44.2 37.5	5.5 11.3	4. 3.	.2 .3	1.5 5.	0 .2
12	Before After	10,650 9,162	50.5 46.8	37.7 29.6	4.2 13.6	0.5 1.5	1. .3	6. 5.1	0 0

eight counts, a decrease in three, and no change in one. Very few eosinophiles were found in any of the counts and no definite change could be noted in these cells. The basophile counts showed an average increase after splenectomy in three rabbits and a decrease in the remaining five.

From the above experiments the following conclusions may be drawn:

1. There is always an anemia produced in rabbits by splenectomy which has a duration of sixty or more days, after which time the red blood count and hemoglobin return to normal.

2. There is no definite change produced by splenectomy in the platelet count and coagulation time.

3. Changes in the total white cell count produced by removal of the rabbit's spleen are not constant. The same conclusion applies to the elements in the differential counts with the exception of the large mononuclears which were increased in every instance after splenectomy.

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# LABORATORY METHODS

## A REVIEW OF MOSENTHAL'S WORK ON THE MEASUREMENT OF THE RENAL FUNCTION\*

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THE work of Mosenthal<sup>1</sup> was suggested by the observations of Hedinger and Schlayer<sup>2</sup> that the response of the kidney to a full diet containing a reasonable amount of fluids, salt, and purins varies quite markedly in health and disease. In these observations the urine voided every two hours was measured, its specific gravity taken, and the salt content determined. From the results it was apparent that the normal kidney reacts in a wholly different manner from one that is the seat of a disease process. It was demonstrated that the normal kidney could excrete a urine of either high or low concentration, depending upon which was necessary at any given time to maintain a normal equilibrium between salts and fluids in the body. In certain diseased conditions of the kidney, on the other hand, this relation became disturbed and there was a tendency on the part of the kidneys to excrete a urine in which the specific gravity and the percentage of salt excretion remained more constant in the two-hour periods. This tendency toward fixation of the specific gravity in certain kidney diseases represents the basis of the test of renal function. Mosenthal has extensively elaborated the method, shown its numerous possibilities and with the renal test meal has supplied a most satisfactory and practical procedure for the study of kidney function. Recently Mosenthal<sup>1</sup> has recorded the results which he has obtained from the study of a great variety of kidney lesions, by the use of a so-called "test meal" as an index of renal function. Our experience with it prompts us to write a review of the work of Mosenthal and to impress the value and practicability of the test meal in all routine studies on suspected kidney diseases. We believe the information derived from the careful use of the test meal is much more extensive than it is possible to acquire from any other method for studying kidney function.

The method can be carried out quite satisfactorily with the expenditure of a very small amount of time. When carefully performed and properly interpreted it undoubtedly represents the most satisfactory procedure for the study of renal function. One is not justified in recommending this method to the exclusion of all others, but when the information which is derived from the test meal is considered along with that gained by the phenolsulphonphthalein test and with accurate clinical observations, much more can be said diagnostically and prognostically than from the use of a single method. It is a test which has a greater range of possibilities than any of the methods based upon the ability of

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<sup>1</sup>Mosenthal: *Jour. Am. Med. Assn.*, 1916, lxvii, 933; *Arch. Int. Med.*, 1916, xvi, 733.

<sup>2</sup>Hedinger and Schlayer: *Deutsch. Arch. f. klin. Med.*, 1911, cxiv, 120.

the kidney to excrete a single dye or a salt. The numerous procedures which depend upon the measurement of the degree of retention of various non-nitrogenous substances in the blood are not as practical because of the necessity of greater technical skill. The kidney is such a selective organ and its functions so diversified that information gained from one angle alone may be grossly misleading.

The basis of the test diet, as elaborated by Mosenthal, depends upon placing the subject to be studied upon a diet sufficiently varied in nitrogenous substances and salt to give an adequate test of the eliminative ability of the kidneys. The diet as recommended by Mosenthal with his complete instructions will be given in full, but if it is not practicable to carry out the test as advised, certain modifications may be made. These modifications will be discussed further along in the paper.

*Directions for the Nephritic Test Meal.*—The following is the standard test meal in use in Lakeside Hospital, which follows that recommended by Mosenthal:

All food is to be salt-free food.

All food or fluid not taken must be weighed or measured after meals and a record kept. No food or fluid of any kind is allowed between meal times.

Mishaps or irregularities that occur in giving the diet or collecting the specimens must be noted.

Breakfast, 8 A.M.:

Boiled oatmeal, 100 gm.; sugar, 1 to 2 teaspoonfuls; milk, 30 c.c.  
Two slices bread (30 gm. each); butter, 20 gm.  
Coffee, 160 c.c.; sugar, 1 teaspoonful; milk, 40 c.c.  
Milk, 200 c.c.  
Water, 200 c.c.

Dinner, 12 Noon:

Meat soup, 180 c.c.  
Beefsteak, 100 gm.  
Potato (baked, mashed, or boiled) 130 gm.  
Green vegetables as desired.  
Two slices bread (30 gm. each); butter, 20 gm.  
Tea, 180 c.c.; sugar, 1 teaspoonful; milk, 20 c.c.  
Water, 250 c.c.  
Pudding (tapioca or rice), 110 gm.

Supper, 5 P.M.:

Two eggs cooked any style.  
Two slices bread (30 gm. each); butter, 20 gm.  
Tea, 180 c.c.; sugar, 1 teaspoonful; milk, 20 c.c.  
Fruit (stewed or fresh), 1 portion.  
Water, 300 c.c.

8 A.M. No fluid or food is to be given during the night or until 8 o'clock the next morning (after voiding), when the regular diet is resumed.

Patient is to empty bladder at 8 A.M., and the end of each period, as indicated below. The specimens are collected for the following periods in properly labeled bottles: 8 A.M. to 10 A.M.; 10 A.M. to 12 N.; 12 N. to 2 P.M.; 2 P.M. to 4 P.M.; 4 P.M. to 6 P.M.; 6 P.M. to 8 P.M.; 8 P.M. to 8 A.M. If specimens are not examined at once, a preservative such as thymol or chloroform should be added, or specimen should be kept in ice box.

The salt is furnished in weighed amounts in three capsules, each capsule containing 2.3 grams of salt. One capsule for each meal.

This meal contains about 3.4 grams of nitrogen, 8.5 grams of salt inclusive of the 2.3 grams given in each capsule, 1760 c.c. of fluid and considerable purins. This diet is given to the patient for one full day, during which the specimens are collected every two hours. Each specimen of urine is accurately measured and the specific gravity determined. The night urine is saved in one twelve-hour specimen and its amount and specific gravity determined. At the termination of the test, the twenty-four-hour urine has thus been collected in six two-hour specimens and one twelve-hour specimen. Nitrogen and total sodium chloride are determined in the total day specimen and the total night specimen, the results being then balanced with the intake. If the diet as here given has been taken in its entirety, the final balancing is much simplified. If it has not all been taken, accurate weights must be had of the portions that were refused. The above diet has been worked out primarily for the use of hospitals where all the necessary chemical procedures can be conducted. If the exact diet can not be supplied in the home, certain modifications may be made without interference with the value of the test, so long as it contains enough salt, nitrogen, and purins to give a proper test of kidney function. In such cases it is well to have the patient record on a slip of paper what was eaten at each meal so that there is no doubt as to the amount that was taken. It is desirable also to keep account of the amount of water and fluids taken at each meal. The urine is collected in exactly the same way as in the directions given above and the amount of each specimen and the specific gravity determined. If patients are irrational and have involuntaries, the mere collection of two-hour specimens and determining the specific gravity will yield very valuable information.

Mosenthal says, "that great variations may be permitted in the above mentioned directions but that certain ones must be followed slavishly, if the maximum results are to be obtained." The urine must be collected punctually every two hours, if it is possible; if not, the urine should be voided at the end of the next two-hour period.

Two or three important points must be kept in mind in the examination of all specimens: the characteristics of the day urine, and night urine, and the specific gravity of each individual specimen; the quantity of the individual specimens of the day urine, and the quantity of the night urine.

#### THE NORMAL URINARY RESPONSE TO THE NEPHRITIC TEST MEAL.

Only one of the tables, showing the normal urinary response to the renal test meal, will be given as it will serve as an example to point out the things to be borne in mind in passing judgment on subsequent reactions, or those showing a deviation from the normal. Usually in normal individuals there is a slight diuresis immediately following each of the meals. This was well marked in the observations of Hedinger and Schlayer,<sup>2</sup> but it has not been so prominent in the normal reactions to the nephritic test meal in Mosenthal's series. In the reaction given in Table I, there is no diuresis following breakfast, but there is a slightly delayed diuresis following each of the other meals.

TABLE I.

## NORMAL

TIME	AMT. C.C.	SP. GR.	SODIUM CHLORIDE		NITROGEN	
			GM.	%	GM.	%
8-10	75	1.017	0.7	.94	1.008	1.34
10-12	261	1.006	1.67	.74	1.196	.46
12-2	530	1.000	1.96	.37	1.44	.27
2-4	194	1.013	1.67	.86	1.227	.63
4-6	135	1.017	1.16	.86	1.036	.76
6-8	395	1.003	1.42	.35	1.327	.34
Total day	1590		8.38		7.234	
Night 8-8	290	1.021	1.68	.58	3.717	1.28
Total	1880		10.26		10.951	
Intake	1760		8.5		13.4	
Balance	-120		-1.76		+1.57	

The important points in this normal reaction are as follows:

1. A night urine of under 400 c.c. in quantity.
2. A positive balance of fluid to the body of not over 400 c.c.
3. Variation of the specific gravity of the day specimens of ten points or more and a night urine with a specific gravity of 1.016 or over.
4. Excretion of all of the ingested salt.
5. An output of 90 per cent or more of the ingested nitrogen through the kidney and a night urine containing more than 1 per cent of nitrogen.

Nocturnal polyuria represents, in the majority of instances, the first manifestation of a failure on the part of the kidney to properly perform its function. As the power of the kidney to concentrate the urine begins to fail, more water must be excreted to rid the body of salts; consequently, a gradual increase in the frequency of urination during the night should in all cases be viewed with suspicion. Mosenthal has shown when normal individuals are being observed for their reaction to the renal test meal, the night urine never exceeds 400 c.c. If, however, an extremely small amount of fluid or food is taken after 5 P.M. the output becomes greater. Of the ingested fluids, by far the largest amount is given off as urine, some, however, is lost by respiration, sweat, and feces, and some is retained by the body to maintain its normal water balance. Normal individuals may show a marked diuresis on this diet and will lose all of the ingested fluids, beside some from their body. As a general rule most normal patients retain with this diet up to 400 c.c. of fluid; consequently, any retention over 400 c.c. should be looked upon as abnormal. The specific gravity in the daily two-hour specimens and night specimen from normal kidneys show a variation in the specific gravity of 10 points, unless water has been unduly restricted. This ability on the part of the kidneys to excrete a urine of either high or low specific gravity, depending upon which is necessary to maintain the normal fluidity of the body, is distinctly an indication of unimpaired renal function. The salt excretion in normal individuals upon this diet is usually slightly in excess of the intake. The reason for this slight salt excess in the excretion is due to the mild diuresis that is produced by the renal test meal. About 90 per cent of ingested nitrogen is excreted

in the urine, the remainder escapes through the sweat and feces; consequently, subjects with normal kidney elimination will have a positive balance of from 1.5 to 2 grams of nitrogen on this diet. The night urine will contain more than 1 per cent of the total nitrogen output, in subjects without any fixation of the specific gravity.

#### THE URINARY RESPONSE TO THE TEST MEAL IN DISEASE.

When the kidney has become the seat of a nephritis, the flexibility of the organ becomes impaired and this impairment is manifested by an inability to concentrate the urine. It must also be borne in mind, that the inability to concentrate the urine is not alone the manifestation of a diseased kidney, but that many other conditions give rise to the same phenomena. Mosenthal has observed fixation of specific gravity in nephritis, pyelitis, cystitis, associated with prostatic hypertrophy, hydronephrosis, pyonephrosis, polycystic kidneys, renal congestion due to cardiac decompensation, diabetes insipidus, and severe anemias.

Table II gives Mosenthal's data of the specific gravity in the specimens obtained in the above mentioned conditions, when patients are put on the test nephritic diet. This is a representative list from the Table as compiled by Mosenthal.

TABLE II.

CASE	SPECIFIC GRAVITY OF SPECIMENS	DEGREE OF VARIATION IN READINGS
Normal	16-19-12-14-20-10	10
Incipient primary contracted kidney	09-14-09-10-14-06	8
Advancing primary contracted kidney	19-20-20-20-21-20	2
Advanced primary contracted kidney	11-11-10-11-11-11	1
Incipient chronic diffuse nephritis	09-16-15-17-12-07	10
Advanced chronic diffuse nephritis	12-11-14-11-13-11	3
Congested kidney; myocardial decomp.	18-20-19-18-20-21	3
Polycystic kidney	10-10-10-11-10-10	1
Marked anemia	10-10-10-10-10-11	1
Diabetes insipidus	04-04-06-04-04-04	2
Cystitis, pyelitis, prostatic hypertrophy	10-10-10-10-11-11	1
Pyonephrosis	11-12-12-13-12-12	2

Table III represents the response of a case in Mosenthal's series, with marked myocardial decompensation, to the nephritic test meal. The urine output is extremely small; the night urine is slightly under normal in amount. There is a marked positive fluid balance, showing that the patient was accumulating an edema. There is fairly marked fixation of the specific gravity; it is, however, fixed at a high point, showing that the kidneys still had power to concentrate the urine. The salt retention here is also quite consistent with the usual findings in

TABLE III.

REACTION TO THE TEST MEAL IN MARKED CARDIAC DECOMPENSATION.

TIME OF DAY	URINE	SP. GR.	SODIUM CHLORIDE		NITROGEN	
	C.C.		%	GM.	%	GM.
8-10	65	1.025				
10-12	53	1.024				
12-2	51	1.024				
2-4	49	1.025				
4-6	37	1.024				
6-8	57	1.021				
Total day	312		0.58	1.81	1.53	4.77
Night 8-8	172	1.021	0.42	0.72	1.67	2.87
Total day	484			2.53		7.64
Intake	995			7.00		9.40
Balance	+511			+4.47		+1.76

this type of case, where there is a rapidly accumulating edema, and the salt is needed to sufficiently concentrate the fluid retained. The nitrogen excretion is perfectly normal.

Table IV shows the reaction of an Advanced Hypertensive Nephritis, in our series, to the Test Meal.

TABLE IV.

REACTION TO THE TEST MEAL IN ADVANCED HYPERTENSIVE NEPHRITIS.

TIME OF DAY	URINE	SP. GR.	SODIUM CHLORIDE		NITROGEN	
	C.C.		%	GM.	%	GM.
8-10	79	1.007				
10-12	145	1.009				
12-2	123	1.009				
2-4	150	1.009				
4-6	115	1.010				
6-8	78	1.007				
Total day	690		.27	1.87	0.36	2.48
8-8	695	1.009	.28	1.92	0.40	2.81
Total 24 hours	1385			3.79		5.29
Intake	1460			5.70		9.11
Balance	+75			+1.91		+3.82

The marked fixation of the specific gravity, the greatest variation being but three points in all of the specimens, and the constancy of the urine output in each two-hour periods, will be observed. There is quite a marked nocturnal polyuria; this is usually one of the earliest manifestations of an hypertensive nephritis, and becomes especially marked in the late case. The salt reaction is perfectly normal. From the large positive nitrogen balance in this case there is evident nitrogen retention, which is very characteristic in the advanced cases of hypertensive nephritis.

Table V shows the response of kidneys that were the seat of a chronic diffuse nephritis. The daily specimens are small in amount, with an evident nocturnal polyuria. There is marked water and salt retention. This patient, like

TABLE V.

REACTION OF CHRONIC DIFFUSE NEPHRITIS TO THE TEST MÈAL. (MOSENTHAL.)

TIME OF DAY	URINE	SP. GR.	SODIUM CHLORIDE		NITROGEN	
	C.C.		%	GM.	%	GM.
8-10	32	1.025				
10-12						
12-2	54	1.024				
2-4	64	1.033				
4-6	64	1.028				
6-8	66	1.030				
Total day	280		0.18	0.50	1.91	5.34
Night 8-8	595	1.016	0.08	0.48	0.93	5.53
Total 24 hours	875			0.98		10.87
Intake	1760			8.50		13.40
Balance	+885			+7.52		+2.53

the preceding one, was accumulating an edema. The concentration of the individual urine specimens is high, and there is a fixation of the specific gravity. In some cases there may be very severe nephritis without such a marked fixation of the specific gravity. It is in the chronic diffuse nephritis that the fixation of the specific gravity is equivocal; for example, in one case of early diffuse nephritis, the following specific gravities were noted: 1.016, 1.015, 1.005, 1.008, 1.015, 1.014, and 1.017. In certain instances, as in this case, if it were not for the clinical evidence that the patient was accumulating an edema, this response to the test meal would have doubtless been hard to differentiate from one which was perfectly normal. The nitrogen excretion in this case is perfectly normal. The most important point to observe is the high nitrogen excretion with a nocturnal polyuria. In the hypertensive nephritis, there is a marked nocturnal polyuria with a low nitrogen excretion, i. e., much under 1 per cent. In the chronic diffuse nephritis the nocturnal polyuria may be quite evident but the nitrogen excretion follows it very closely in trying to maintain a 1 per cent average.

The response to the nephritic test meal in the case of chronic diffuse nephritis may simulate very closely that obtained in a myocardial decompensation. The main points of differentiation are the physical examination, the nocturnal polyuria, and the fixation of the specific gravity.

The cases with myocardial insufficiency have no nocturnal polyuria, and the specific gravity is usually fixed at a high point. The chronic diffuse nephritis has marked nocturnal polyuria and may or may not have fixation of the specific gravity, but if it is fixed it is usually at a lower point. Mosenthal has shown that myocardial decompensation cases vary in their reaction to the test meal, depending upon whether they are decompensating with an accumulating edema, or whether the edema is disappearing, or shortly after it has disappeared.

## SUMMARY.

The test as carried out represents a study of specific gravity, salt, nitrogen, and water excretion on two-hour specimens during the day and twelve-hour urine for the night period. Normal individuals yield urine specimens which vary 10

points or more in the specific gravity. The volume of the night urine never rises above 400 c.c. The specific gravity is usually 1.016, or above, and the nitrogen excretion over 1 per cent. When kidney function becomes impaired it is first manifested by a rise in the excretion of the night urine; i. e., the night urine exceeds 400 c.c. and the nitrogen excretion becomes lower than 1 per cent. In severe cases of chronic nephritis the specific gravity becomes markedly fixed both in the day specimens and the night urine. The fixation of the specific gravity usually occurs at a very low level. Such changes are not alone confined to nephritis, but may occur in other conditions; pyelitis, cystitis, hypertrophied prostate, marked anemia, pyonephrosis, polycystic kidney and diabetes insipidus. It will appear evident that the cause of the deviation from a normal reaction to the test meal must be sought in either the blood, kidneys, or the urinary passages.

Finally the test meal represents a rather elaborate study of many phases of kidney function. However, it is not a complicated procedure, and is one that lends itself very nicely to the needs of the busy practitioner.

## A THERMOSTAT FOR A WATER-BATH

BY MILES J. BREUER, M.D., LINCOLN, NEB.

**I**N a past issue of the Journal, the writer described a thermostat for a laboratory water-bath for the inactivation of vaccines and serums and the incubation of biologic reactions. The thermostat consisted of capillary tubes and bulbs containing mercury; at the proper degree of temperature the mercury made a contact with a platinum-pointed wire, thereby operating a relay which broke the circuit of the heating element. This thermostat was very accurate when in good order, but had the following disadvantages: it was difficult of construction for one not experienced in glass working and electrical work; it was very complicated, easily got out of adjustment, took up a large amount of space, and the adhering of the mercury to the sides of the capillary tube and consequent interference with accuracy was very difficult to avoid. The writer has, therefore, worked with a number of different forms of thermostat, with a view to finding one which is readily made, compact, and not easy to get out of adjustment. The two described here have been found, upon use for some time, to fulfill these requirements.

The first one that was found serviceable is illustrated in Fig. 1. It consists of a two-ounce flat ointment can of tinned sheet iron, a very familiar object to the drug dispenser. The lid was soldered on around the entire circumference, so that the can was completely sealed, and capable of withstanding some internal pressure. A few cubic centimeters of ether are sealed in the can, being introduced through a pin-point opening which is then soldered up. This is done after the rest of the soldering on the instrument is finished, but is mentioned first in order to make the principle clear.



The warming of the drum vaporizes the ether and creates a pressure, which raises the upper surface of the drum, raising in turn the upright *C*, lifting the contact at *H* from the fixed arm *BDG*, and breaking the heating circuit. *B* is a heavy piece of brass soldered to the side of the drum, and bent at right angles at *F*. At *F*, a piece of heavy red fiber material *D* (such as is used by surgeons for splints or by electricians as an insulator) firmly riveted, so that it is parallel to the top of the drum, and its end extends over the central point of the upper surface of the drum. On the end of the fiber piece, at *G*, is riveted a piece of

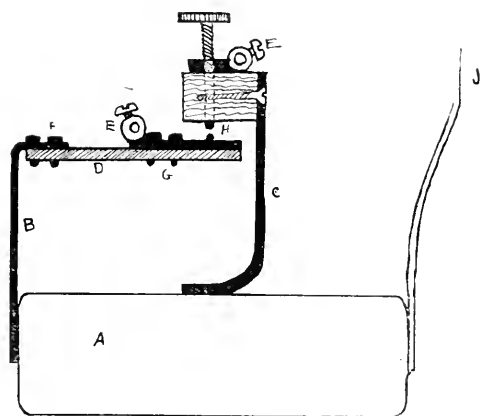


Fig. 1.

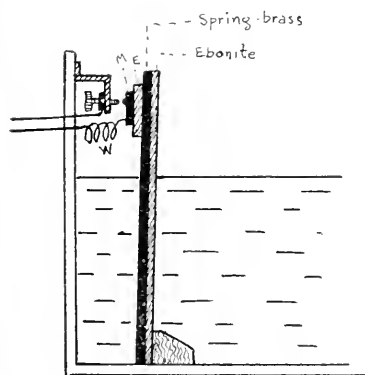


Fig. 2.

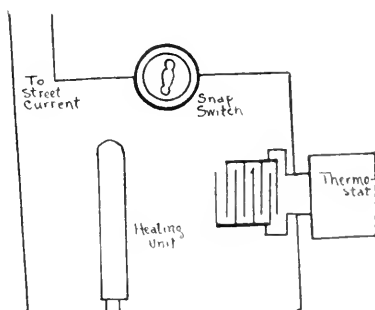


Fig. 3.

brass, which carries the platinum contact point, and the wire-connector, *E*. The upright piece, *C*, is soldered by its bent base to the central part of the drum's upper surface. To its upper end is screwed a block of oak (or of some insulating composition); and through it is put the contact screw with milled head for thumb adjustment. It is preferable that the thread of the screw catch in the block, so as to hold it steadier. This makes the adjustment more accurate. Above the block, a nut is threaded on the screw, carrying the wire-connector, *E*. The purpose of the block and the fiber strip is to prevent short-circuiting, and grounding of the current in the water. The piece *J* was soldered on the drum in order that the instrument may be firmly attached to the side of the containing

vessel. The entire apparatus was given a heavy coating of copper in an electric bath, to prevent corrosion in the water-bath.

Another thermostat which was found to be simpler and more easily constructed, though not quite so accurate, is shown in Fig. 2. It consists of a bar of two substances with different coefficients of heat expansion, fastened rigidly together, fixed vertically to the bottom of the vessel containing the water. At first we used a bar of spring-brass and ebonite, riveted together with numerous rows of rivets, placed as close together as possible. Later, a bar was made by soldering together a strip from a large clock-spring, and a piece of copper. Both forms worked equally well. The upper end projects from the water, so that the portion where the connections and contacts are made, is not in danger of becoming wet. A block of ebonite or fiber *E*, is riveted to the end of the bar to furnish an insulating base on which to mount the contact and thereby prevent grounding of the current in the water. The wire is led to this, through a loose coil of soft wire, *W*, in order that the motions of the bar may not be interfered with. The platinum contact is carried on a small plate of brass on the surface of the fiber or ebonite, to which plate the connecting wire is also soldered.

The contact screw is mounted as shown in the illustration, on a bracket of ebonite, fiber, or hard wood, or some insulating material, to prevent grounding of the current.

Both of these pieces of apparatus are easily constructed. If the laboratory worker is not able to do the soldering and riveting himself, a machinist will do it for him at a very small cost. The copper-plating required in the case of the apparatus of Fig. 1, is a very simple procedure for a laboratory furnished with a source of direct current, or, a plater will do it for a few cents. The convenience afforded by the possession of an apparatus of this sort will more than repay even the small laboratory for the trouble or cost entailed in its construction. Fig. 3 shows the connections recommended for the thermostat and water-bath, including a condenser to prevent excessive sparking at the point where the contact is broken.

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## EDITORIALS

### *The Composition of the Alveolar Air and the Respiratory Dead Space*

THE importance of the estimation of the gaseous composition of the alveolar air is emphasized by the numerous recent papers in which the tension of the alveolar gases forms a basic figure in the data from which the conclusions are drawn. One has only to consider what a hair-trigger mechanism the respiration is to appreciate what gross changes may take place from moment to moment in the air within the lungs. When we swallow, talk, perform a precise movement, or when the attention is directed to the respiration, we unconsciously change the type of breathing. This affects the composition of the alveolar air to a greater or less extent. The theoretic factors which must be considered in a method to estimate the composition of the alveolar air are easily determined, but to produce a method which conforms to the theoretic requirements and at the same time is practical because of the personal factors, is one of the most difficult problems which confronts the physiologist.

The simplest method which has been devised to determine the tension of the alveolar gases is that of Haldane and Priestley, which consists in having a

person with his nose clamped breathe through a piece of hose pipe about a meter long, furnished at the end next the mouth with a side tube leading to an evacuated gas-sampling tube of about 50 c.c. capacity. After the person has become accustomed to breathing through the tube, he is asked to make a forced expiration and at the end of it to close the mouth-piece with his tongue. At this moment the operator immediately opens the tap of the sampling tube and air rushes in from the tube through which the person has expired. This sample evidently represents air which has been in the alveoli. Its percentage composition is then determined by analysis. Since the tension of the alveolar gases changes on account of the diluting effect of the inspired air, it is necessary to take two samples, one from the expiration following a normal inspiration and one from a forced expiration following a normal expiration. The average of the percentages found for each gas is supposed to give the mean percentage of the gas in the alveolar air.

On account of the difficulty in having patients, especially children, assist properly in this method, others have been devised, of which the simplest perhaps is that of Fridericia, which consists in expiring forcibly through a specially constructed U-tube and then analyzing the sample of the air remaining in the tube. The method has been described fully in an earlier number of the Journal. Henderson and Morriss<sup>1</sup> have recently proposed an excellent modification of the Haldane-Priestley method for obtaining the alveolar sample. In the latter method they point out that one of the chief difficulties lies in the fact that untrained subjects are apt to take a deep inspiration before they make the forced expiration. In order to obviate this difficulty, Henderson and Morriss, have devised an instrument, which consists of a glass tube of moderate bore at the lower end and of small bore at the upper end, with a small bulb connecting the two tubes. The lower end fits loosely into a large-necked bottle containing a little acidulated water. The upper tube is connected with a mouth-piece by a small rubber tube, which can be closed with a Mohr clip. The subject is instructed to take a normal breath through the tube and then to expire forcibly. In doing this he sucks a little water into the tube, but since the bottle contains insufficient water to fill the tube, air is sucked in with some resistance. This slight resistance and the bubbling of the air passing through the water tend to prevent the subject from taking more than a normal inspiration; and when the forced expiration is made, the last part of it is caught by closing the upper end with the pinchcock, the water automatically closing the lower end. The air in the tube is then analyzed.

Another method which has been extensively used for clinical purposes is that of Plesch, which consists in having the person breathe several times in and out of a bag. It is assumed that after such respiration the composition of the air in the bag will become the same as that in the alveoli. Although there is no doubt that this is true, yet it has been shown that the method is fallacious on account of the fact that the carbon-dioxide tension thus determined is not that of the arterial blood, but rather approaches that of the venous.

The principle used in the Haldane-Priestley method, and in its various modifications, has been severely criticized by Krogh and Lindhard.<sup>2</sup> These authors

point out that the time required to expire forcibly is appreciable, and that during this time the percentage composition of the alveolar air is changing. Therefore, a sample taken from an expiration following a normal inspiration does not represent the alveolar air at the end of inspiration, but at a time somewhat after that, when the oxygen tension is highest and the carbon-dioxide tension lowest in the alveolar air. The average obtained from the estimations of samples taken at the end of a forced expiration following a normal inspiration and expiration is, therefore, higher than the mean tension for carbon dioxide. They point out that this error is exaggerated when the rate of metabolism is increased, as during exercise, and they account in this way for the great increase in the capacity of the air passages found by Haldane and Douglas during the deep breathing of exercise. For this reason they believe that it is much better to calculate the composition of the alveolar air from the average composition volume of the expired air and the volume of the dead space, the latter figure, they assume, being fairly constant for persons of the same height and weight. In this view they are directly opposed to the teachings of the Haldane school that the dead space is a rapidly and constantly changing volume, and not an anatomic entity. Haldane and co-workers, in England, and Henderson and his pupils, in this country, did not accept the criticism of Krogh and Lindhard, and they again championed the theory that the "virtual" or "physiologic" dead space varies with the size of the respiration, and that, therefore, all estimations of the tension of the alveolar gases based upon a constant dead space are of doubtful accuracy, and that the dead space is not an anatomic structure. Indeed, Haldane and Henderson both affirm that the dead space progressively increases up to over four hundred per cent in breaths of increasing volume until the maximum, and that the virtual, or physiologic, dead space for carbon dioxide is less than that for oxygen. They account for this by assuming that the carbon dioxide is excreted by the bronchial tubes more rapidly than oxygen is absorbed. They also answered the criticism of Krogh and Lindhard in part by showing that in deep breathing alone the dead space is increased exactly as much as during exercise, and that, therefore, the error which increased metabolism introduces into the alveolar air determinations is not of vital importance.

Krogh and Lindhard have recently repeated their earlier experiments and have confirmed their conclusions that the dead space does not vary appreciably when respirations of ordinary volume are made, and that only with the deepest respirations is the increase in the capacity of the air passages important. For example, they found that the increase in the dead space when respirations of 1500 c.c. were made was slight, and that respirations of about twice this amount increased the capacity of the dead space by perhaps 100 c.c. This increase they believe to be due to the stretching of the bronchial tubes. They point out that the difference in their results and those of Haldane and Henderson in regard to changes in the capacity of the dead space is due to an error introduced by the fact that one of the basic figures in the formula which Haldane and Priestley use in the calculation of the dead space is the percentage of carbon dioxide excreted or oxygen absorbed in the lung air, which figure is obtained by the Haldane-Priestley method outlined above, and is accordingly higher than it should be.

The small increase in the size of the dead space which Krogh and Lindhard found to occur during progressively increasing respiratory volumes, is of some interest. Their data show that the increase, small as it relatively is, becomes progressively greater as the volume increases. With respirations of moderate depth there is only a small increase in the capacity of the respiratory passages, and beyond this point the change becomes progressively greater. These investigators believe that their results show that the dead space is a real anatomic entity, consisting of what one may term the nontranspiratory portion of the lungs, that is, the upper respiratory passages and bronchioles. The increase they find in deep respirations they attribute to stretching of the bronchioles. A consideration of the data, which they plot as a curve, is, interesting from a mathematic standpoint, since it lends force to their argument. The walls of the bronchi and bronchioles are made up largely of elastic and smooth muscle tissue, which is extensible and responds to changes in pressure with equal degrees of expansion or contraction. As the respirations increase, the expanding force of the inspiratory movements of the thorax increases, the diameter of the bronchi and bronchioles enlarging by more or less equal increments according to the amount of the expanding force. The increase in the diameter or circumference is directly proportional to this force, but the area of the cross section of the bronchi increases progressively as the square of the diameter. This is more easily appreciated if one calls to mind the fact that the area of a circle is increased about 100 per cent when its circumference is increased by 50 per cent, and about 400 per cent when its circumference is increased by 100 per cent.

With this fact in mind, the relative constancy of the dead space when the respirations are relatively small is at once explained, and the small progressive increase observed by Krogh and Lindhard is, from a mathematic, as well as a physiologic, standpoint, to be expected. However, Henderson<sup>1</sup> in a very recent paper still clings to the belief held by the English school, that the dead space is not an anatomic entity and that it is subject to great variations, and this latter point he considers Krogh and Lindhard to have confirmed. In this he is wrong, for a 300 per cent increase in the value of the dead space found by Henderson with maximum respirations can not be interpreted as being confirmed by the 66 per cent increase found by Krogh and Lindhard for maximum respirations. What is more important, however, is the fact that Henderson does not concede the point which Krogh and Lindhard make regarding the error in the Haldane-Priestley method for obtaining samples of the alveolar air, and it is upon this point that the difference between the Haldane and the Copenhagen schools rests. Krogh and Lindhard determine the capacity of the air passages indirectly by the use of an inert gas, such as hydrogen. Haldane believes that this method is not allowable. A few months ago, therefore, the question stood unsettled, the English school, championed here by Henderson, believing the Haldane-Priestley method for determining the gaseous composition of the alveolar air to be fairly accurate and capable of use in determining the reactions of the respiratory center and the capacity of the dead space; the Danish school, on the other hand, believing it to be unreliable and especially so when the subject is untrained and is not completely resting and breathing normally.

It is a matter of some importance that this question be settled, as many most important physiologic and pathologic teachings have their foundation in the determination of the percentage composition of the alveolar air as determined by any of the numerous methods. Probably the Haldane-Priestley method is accurate enough for many types of investigations, but its personal and theoretic error is large, and any figures obtained by it are not capable of being interpreted save in a gross manner. To be sure, Haldane and his coworkers have demonstrated by its use that there is a fair degree of constancy in the pressure of the alveolar gases under constant physiologic conditions, and gross changes have been shown to occur when the physiologic state of the body is changed as in exercise, at high altitudes, or in some diseases. However, when fine differences are sought, such as are required in studying the sensitivity of the respiratory center or the capacity of the dead space under various conditions in which the changes in the alveolar air are not gross, the Haldane-Priestley method is certainly disappointing, and it contains other theoretic errors besides that pointed out by Krogh and Lindhard, as will be shown below.

A moment's consideration of the theoretic changes taking place in the alveolar air during the various phases of respiration reveals some very important and hitherto little emphasized facts, and it also reveals the source of some errors in the methods of alveolar air analysis. Krogh and Lindhard have settled beyond question the fact that the partial pressure of carbon dioxide in the blood leaving the lungs and that in the alveolar air are practically identical. Barcroft has shown that the hemoglobin of the blood is practically saturated with oxygen at partial pressures of 65 to 70 mm., and that over 90 per cent of the oxygen is carried by the blood in this chemical combination. Bohr and, later, Christiansen, Douglas and Haldane have shown that the combination of carbon dioxide with blood is greater at low than at high partial pressures of carbon dioxide, this being brought about by the fact that carbon dioxide is held both in solution and in chemical combination in the blood; and, furthermore, blood will hold a greater amount of carbon dioxide under a given pressure of carbon dioxide in the absence than in the presence of oxyhemoglobin, the difference being proportional to the amount of the oxyhemoglobin present.

With these facts in mind, let us consider the changes which occur during a respiratory cycle. The cycle consists of two approximately equal phases, inspiration and expiration. During the cycle the blood delivers a certain amount of carbon dioxide to the air and in turn takes up a certain amount of oxygen from the air in the alveoli. The maximum fall in carbon-dioxide pressure in the alveolar air occurs during inspiration, for it is during this time that the whole diluting atmospheric air is taken into the lungs. Since the evolution of carbon dioxide is relatively more rapid at low than at high partial pressures of carbon dioxide, it is clear that the greater amount of carbon dioxide is given off by the blood while the carbon-dioxide pressure is falling during inspiration, and that each volume unit of carbon dioxide produces less change in the pressure of the gas in an increasing volume of alveolar air than it does when delivered to a preceding volume, as is the case during the expiratory phase. In order that the volume of carbon dioxide actually given off by the blood to the alveolar air might be the

same during inspiration and expiration it would require the delivery of equal amounts of the inspired air to the alveoli during both phases of the respiration. Since this is not the case, it follows that the volume per cent of carbon dioxide held by the blood leaving the lungs during inspiration is less than that held during expiration. The maximum pressure of carbon dioxide in the alveolar air is present shortly after expiration and the minimum at the end of inspiration. Because the evolution of gas is greater in amount and more rapid at low pressures than at high pressures of carbon dioxide, the mean pressure of carbon dioxide in the alveolar air is reached some time before the midpoint of expiration and after more than half of the carbon dioxide excreted during expiration has been evolved.

From the above argument it is clear that the amount of carbon dioxide lost by the blood during inspiration is greater than that given off during expiration, and a larger fraction of the carbon dioxide is excreted at a pressure below mean than above mean. Any method, therefore, which attempts to estimate the mean per cent of carbon dioxide in the alveolar air must be based upon a principle which takes into consideration the unequal rate of carbon-dioxide excretion during expiration. A sample of air taken at any point of an expiration can not give a correct value of the alveolar air save for the moment of sampling. Nor can averages be taken, as is done by the Haldane-Priestley method. Such procedures, no matter how perfect the technic may be, give values considerably higher than the mean tension of carbon dioxide in the alveolar air.

The case of oxygen, however, is different, for the absolute amount of oxygen which the blood holds when leaving the lungs is approximately constant, since under all ordinary conditions there is more than sufficient oxygen pressure to saturate fully the hemoglobin of the blood. The error Krogh and Lindhard point out as present in the Haldane-Priestley method for the estimation of carbon dioxide with regard to the time element will tend to make the oxygen percentage too low. The ratio of the volume of carbon dioxide excreted to the volume of oxygen absorbed by the blood, in samples of air taken at any time during the expiration, will accordingly be less than that obtained from a mixed sample of the expired air.

In this fact lies the explanation for the Haldane-Henderson view that the dead space for oxygen is greater than that for carbon dioxide. The oxygen absorbed is one of the basic figures in the dead-space determination for oxygen, and is calculated from the difference in the oxygen percentages in inspired and in expired alveolar air. Since it is necessary to know the percentage of carbon dioxide in the sample to obtain the correction required to be added to the percentage of oxygen in atmospheric air actually inspired (a procedure necessary because the amount of carbon dioxide excreted is less than the volume of oxygen absorbed), the error present in the carbon-dioxide estimation is added to that of the oxygen, and the amount of oxygen absorbed is unduly large when compared with that of carbon dioxide. This error gives a low respiratory quotient in samples of alveolar air taken by the Haldane-Priestley method, and since the oxygen dead space is computed by the use of the figure for oxygen absorbed, the oxygen dead space is larger than the corresponding carbon dioxide dead space. The respira-



tory quotients obtained from the analysis of the expired air and from the mean of the alveolar air must be equal, as otherwise we should lose carbon dioxide or gain oxygen. If such is the case, the mean dead space for carbon dioxide and for oxygen must also be equal.

With the above points in mind, Pearce has attempted to devise a method for obtaining the gaseous composition of the alveolar air. He assumes that if an expiration of infinite amount could be made and collected, but occupying the time of a normal expiration and following a normal inspiration, the total expiration would be made up of alveolar air, and the carbon-dioxide and oxygen percentages found in it would be the mean present during expiration, for in it the diluting effect of the dead-space air would approach zero. An expiration of less than infinite amount, however, would contain atmospheric air from the dead space which would reduce the percentage of carbon dioxide in the expired air below the average present in the alveolar air.

Making use of the fact that this reduction is in direct proportion to the amounts of alveolar air present in the expiration, it is possible, by comparing the percentage gaseous compositions of expirations of different volumes, to determine the capacity of the respiratory passages and the mean gaseous composition of the alveolar air. In collecting such expirations, however, the greatest care must be taken to see that the expirations, be they large or small, follow a normal inspiration, and take the same time as is required for a normal expiration, and, what is most important, that the same relationship exists between the carbon dioxide excreted and the oxygen absorbed as exists in the average normal expired air at the time. The method will be fully described in a coming issue of this Journal. Making use of the mathematic principle involved, Pearce has recalculated data given by Haldane and Douglas and by Haldane which had been interpreted as showing a great increase in the capacity of the dead space during deep breathing, and fails to find any evidence that this is the case, or that there is a difference in the volume of the dead space for oxygen and that for carbon dioxide. His results show that the mean percentage composition found in the alveolar air by Haldane's method is from 8 to 13 per cent too high for carbon dioxide, and from 12 to 20 per cent too high for oxygen. These studies confirm the view of Krogh and Lindhard that the dead space is an anatomic entity and subject to only relatively small variations. They fail to confirm the views of Haldane and Henderson regarding the capacity of the dead space in deep breathing and the difference in the size of the dead space for carbon dioxide and for oxygen.

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<sup>3</sup>Pearce: *Am. Jour. Physiol.*, 1917, *liii*, 73; and papers in press.

—R. G. P.

### *Cutaneous Reactions To Various Drug Applications*

SINCE the time to which the memory of man runneth not to the contrary localized skin lesions, caused by the bites or stings of insects, by contact with poisonous plants or chemicals, or, occasionally the more generalized forms of cutaneous eruptions, urticaria, etc., have been a matter of interest to the human race. Much obscurity has at all times surrounded the nature of the processes, or the causes, by which a large number of these lesions have been produced. Often no etiology could be found, and in these cases the tendency has usually been to ascribe the observed lesions to the introduction into the body of some substance, possibly of protein nature, which in some more or less obscure manner produced the results in question. Various investigators have from time to time taken up this problem, and among these the latest have been Sollmann<sup>1</sup> and Pilcher<sup>2</sup> at the Western Reserve University Medical School.

These observers have performed a long and interesting series of experiments both on man and on animals. The immediate object was to study the effects of iodism on the response to irritants when these substances were applied directly to the scarified skin. But in the course of this work so far a number of important observations have been made which are not immediately concerned with the effects of iodism. Two methods of investigation were used by Sollmann and Pilcher. The first consisted in simply scarifying the skin over a small area on the inner surface of the forearm after which the drug was applied and gently rubbed in for a minute or so with a glass rod. The technic was similar to that of bloodless vaccination. In the second method a very small quantity of dry mucuna was gently rubbed on the skin, after which a drop of the solution to be tested was applied and rubbed in lightly for about one minute. Mucuna ("cowhage," "cowitch") consists of the stiff hairs from a leguminous seed-pod. The hairs puncture the skin and this leads to considerable itching and probably some rubefaction, but no edema. If the solution of an urticarial agent is rubbed on after the mucuna, the skin soon begins to swell around each minute puncture, the wheals growing rapidly and fusing if they are close together. In later experiments Sollmann<sup>3</sup> has scarified the skin by rubbing with sandpaper, carborundum stone, or pumice stone, or by puncturing with a needle (Hill's method<sup>4</sup>) before the drug was applied. In all cases the action of the drug to be studied was controlled by other areas which were scarified or treated with mucuna, but to which no drug solution was applied.

The positive or negative action of the drugs tested was indicated by the character of the skin reaction, those drugs which were practically negative, or entirely negative, producing only a few widely separated minute papules, or no papules at all. Drugs which were slightly positive produced fairly numerous papules, but of small size (2 mm.), which, therefore, did not tend to fuse. Strongly positive drugs produced numerous papules of considerable size (up to 3 or 4 mm. or larger) which tended to fuse into large wheals. The close resemblance of these last lesions to clinical urticaria will be evident at once. These local, endermic, drug reactions, therefore furnish an opportunity to study experimentally certain

features of the probable nature of urticaria. This condition has often been shrouded by a great deal of obscurity regarding both its real etiology and its pathology, as well as in respect to any thoroughly scientific and satisfactory mode of treatment. A peculiar and significant interest, therefore, attaches to any experimental knowledge which may be acquired along these lines. It was found by the use of a long series of drugs that the reaction which any given substance would produce could not be predicted beforehand, but could only be determined by actual test. And in numerous instances wholly unexpected results were obtained. Since only a small local area of the skin was involved it is obvious that generalized systemic reactions through the central nervous system, the digestive system, the vascular system, etc., were not concerned, thus eliminating some of the obscuring features which are often considered to be involved in the production of clinical urticaria. It is also of interest to note that none of the reactions in man or in animals was modified in any way by the administration of iodides.

It was found that certain drugs which produced urticaria locally were also in the group of substances which, in certain individuals, produce it clinically. This was especially true for histamine, morphine, and formaldehyde; and atropine should perhaps also be included in the same group. Another group of drugs, however, which frequently produce clinical eruptions were found to give, *in the subjects tested*, practically negative results. Among these drugs were the salicylates (especially aspirin), phenacetin, antipyrine, quinine, turpentine, copaiba, santal, chloral, and arsenic. Possibly in certain individuals some of these drugs might also produce reactions in the nature of urticaria when they were applied to the scarified skin. This point is well worth trying out clinically. Other drugs which also produced urticaria were calcium chloride (10 per cent solution), urea (strong solution), peptone, phenyl-ethylamine, urethane, etc. It was especially significant that the six most active drugs in the entire series had practically nothing in common, either chemically or pharmacologically and the reaction could only be determined by actual trial. On the other hand there was a rather frequent, but generally feeble, urticarigenic action shown by substances containing the  $\text{NH}_2$  group. But there were many exceptions, and those did not seem to bear any relation to the chemic structure.

The authors adduced evidence that these localized lesions, which usually lasted from a few minutes up to one or two hours, were not due to a localized acidosis, to osmosis, to local vasoconstriction nor to vasodilatation. Nor was sensory irritation the cause, for neither veratrine nor aconite produced the lesions. Inflammatory irritation in general was not the cause, for urticaria was not produced by such irritants as mustard, cantharides, alcohol, saponin, acids, alkalis, metals, etc. Capillary poisons, such as arsenic or uranium, do not produce urticaria, at least in acute experiments. Nor does it appear that altered water affinity or altered patency of the lymph channels could cause the phenomena. It seems possible, however, that a specific increased permeability of the capillary or lymph vessels may be concerned. Several distinct groups of sensory actions were distinguishable, such as the itching of the urticarial agents, the smarting and burning of the simple irritants, the formication of aconite, and the lancinating pain of veratrine.

The rapidity of the formation of these lesions and their brief duration are in striking contrast to the phenomena observed recently by a considerable number of observers<sup>5</sup> in England. In these instances many persons became afflicted with skin lesions over the hands, face, feet, or rarely on other parts, as a result of coming in contact with a yellowish powder contained in, or attached to, bombs which had been dropped during aeroplane attacks on the various localities. In some cases contact with the fumes or vapors in the immediate neighborhood of a bomb explosion appeared sufficient to cause the lesions. The yellowish powder appeared to resemble picric acid, while the skin lesions bore a considerable resemblance to those produced by trinitrotoluene. It appears probable, however, the powder is hexa-nitro-diphenyl-amine.

The cutaneous lesions consisted of a peculiar form of dermatitis which had a latent period varying from four or five up to about nine days from the time of the exposure. The great majority of the cases developed on about the eighth day. From about the first day after the exposure a deep yellowish or orange discoloration of the skin appeared, usually most marked on the hands. A majority of the patients were soldiers who had been engaged in digging unexploded bombs out of the ground into which they sank six or seven feet sometimes. Thus the hands were especially exposed. The orange staining of the hands, face, feet, etc., came on the next day, and about five or six days later the eruption began. The staining persisted, but the hands became swollen, inflamed and edematous, the skin felt hot and tense and there were deep-seated vesicles between the fingers and on the dorsum. In many cases there was intense itching and irritation which was usually worse on the hands. One observer noted that after five weeks the staining on the face and feet had disappeared, but the hands were then peeling off, the flakes being stained and thickened. The new skin under the flakes was tender and red. Constitutionally there were no outstanding symptoms, but there was a slight nausea<sup>6</sup> and anorexia, with some alteration of the taste which was compared to the sensation noticed when chewing tobacco. In other cases the eruption<sup>7</sup> reached its acme in four to five days after the vesicles began to appear. There was no inflammatory redness but only the clear "sagograin" vesicles, characteristic of cheiropompholyx, which appeared deeply under the thick epidermis, rapidly enlarged and ran together to form large blebs, in some instances as large as a hen's egg. These vesicles, large and small, covered the palmar surface and sides of the hands and fingers and the interdigital spaces in all cases, and in two-thirds of the cases similarly affected the soles and toes. In some of these cases the backs of the hands and feet were swollen, but other parts of the skin were not involved. In the course of a week most of the vesicles had dried, and the horny layer was beginning to separate and to leave a new, pink, healthy epidermis. All these patients suffered severe pain, so severe that they were kept awake at night by it. In none of the cases was there suppuration, and this perhaps was due to the treatment which consisted in soaking the hands and feet for half an hour several times a day in hot water. This also eased the pain. After the soaking calamine<sup>8</sup> lotion was applied.

These cases suggest that the eruption known as pompholyx, dysidrosis, or

cheiropompholyx, and which has generally been regarded as due to irritation from toxic sweat, and to be in some way associated with debility from nervous or other influences, may in reality be due to some external poison<sup>9</sup> which gets into the sweat pores and there produces irritation. Thus we may be dealing here again with another of the peculiar and obscure ways in which drugs may affect living tissues, often with the most striking resemblance to the effects produced by disease.

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—D. E. J.

### *Lymphocytes and Tuberculosis*

A DIAGNOSTIC test of value for the earlier detection—two weeks instead of a possible six—of tubercle bacilli in urine (or in sputum when unrevealed by the microscope) has been introduced by Morton and Murphy<sup>1</sup> and quite recently confirmed by McGrath.<sup>2</sup>

This test is one of the first practical results to emanate from the long studies which have been followed on the relationship of the lymphocytes to tuberculous infection.

Murphy and Ellis,<sup>3</sup> in 1914, showed that white mice submitted to x-ray exposure became less resistant to the inoculation of bovine tubercle bacilli. They argued that the destruction of the lymphocyte elements (12,000 white corpuscles of which 40 per cent were lymphocytes were reduced to 4,000 at the expense of the lymphocytes) accounted for the lowered resistance of the mice to tubercle infection.

Bartel<sup>4</sup> was one of the first investigators to call attention to the defensive action of the lymphocyte to tubercle infection although almost constant presence of the lymphocytes in tubercle formation had long been known. Opie's<sup>5</sup> investigations also suggested the importance of the lymphocyte defense. Marie and Fiessinger<sup>6</sup> made the discovery that the lymphocytes contain a ferment (lympholipase) capable of splitting wax and fat into glycerine and fatty acid. Since 30 per cent by weight of the body of the tubercle bacilli is composed of waxy substance, the bearing of the lympholipase can be understood.

It would appear that the effect of x-ray exposure on an animal would depend upon the dosage administered. Small doses will stimulate the production of more lymphocytes, whereas large doses or frequently repeated x ray exposures will reduce and destroy the lymphocytes.

The injections of urine suspected of containing tubercle bacilli must be made into the peritoneal cavity and not subcutaneously because in the former situation the few bacilli present will probably multiply more quickly and before the response of lymphocyte proliferation which is apt to follow the lymphocyte destruction. Experiments will, no doubt, be carried on to find out whether the x-ray in small repeated doses can increase the resistance of laboratory animals to tubercle infection. Webb and Williams,<sup>7, 8</sup> following their discovery of the increased production of lymphocytes at a high altitude, were able to show that artificial hyperemia of the marrow in rabbits would cause a decided lymphocytosis and also an increased protection to bovine tubercle infection.

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—G. B. H.

### *The Paraffin Wax, or Closed, Method of Treatment of Burns*

**D**R. WILLIAM O'NEILL SHERMANN, of Pittsburgh, Pa., has reported to the National Research Council the results of his experiments in the treatment of burns. An abstract of his report may be stated as follows:

Sixteen years ago in China, Barthe de Sandfort first applied paraffin wax in the treatment of burns. The results were so satisfactory that he finally placed on the market, a paraffin preparation under the trade-name of Ambrine. Five years ago, this preparation was introduced into the United States, but received no encouragement. It was not until the present war that de Sandfort had the opportunity of treating a large number of cases. The method of treatment now adopted is as follows:

All burns, regardless of character, are entirely dried and an air-tight coating of paraffin wax is applied to the burned area and extends over the adjoining skin for about one inch. A thin layer of cotton is then applied to the first film of paraffin and a second application made. All burns are redressed once in twenty-four hours. This method does not provide any specific curative medical substances, but acts altogether mechanically. The first cotton dressing is a non-adhesive shell which excludes the air. Ambrine is a neutral paraffin made of beeswax and a resin. It is a secret preparation. In this country there are at present many imitations including Cerelene, Parresine, Redintol, Stanolind, Mulene, Parowax, Hull's Formula No. 7, de Arcy Power's Formula, etc. Shermann finds the first three of the above mentioned substances superior to the others. Cerelene

is made in three forms: A, plain wax; B, beeswax with nine hundredths per cent picric acid; C, beeswax with two per cent of oil of eucalyptus. The advantage of Cerelene over Ambrine and Parresine is that it contains enough beeswax to dissolve picric acid and the other mentioned substances. Paraffin is not as flexible or ductile as Ambrine or Cerelene, which also contains a small amount of acid. Redintol is chiefly paraffin with a small amount of resin and has very much the same physical and chemical properties as paraffin. Shermann recommends that the wax be applied to the burn with an atomizer, and he states that the atomizer manufactured by Harvey R. Pierce, Chestnut Street, Philadelphia, is the best that has come to his hand.

The details of the treatment are stated as follows: When the patient enters the hospital the clothing is removed; blebs are punctured, but not excised, and the entire area entirely dried with a Shelton electric hot-air draft, or a Hamilton-Page fan, so that there is no moisture left on the surface. An electric fan, or even a common fan may be used. The wax is applied to the burn at the earliest possible opportunity either with a fine varnish brush or an atomizer. It is difficult to regulate the temperature when the brush is used, besides the application with a brush is more or less painful and there is a tendency to brush away and injure the new epithelium and granulations. If a brush is used, the wax should be gently dabbed on without pressing: to and fro painting movements are painful and should never be used. The wax can be sprayed on with an atomizer without pain or discomfort, and can be more evenly distributed. The atomizer is doubly jacketed—a jacket keeping the wax liquid for thirty minutes after it has been heated, and it may be sprayed either with a handle, a compressed-air pump, or air tank. The wax is placed in the atomizer and it, in turn, placed either in an autoclaving sterilizer, or on a hot plate. Usually ten minutes' boiling is sufficient to bring the wax to a proper liquid state. One must be careful to prevent the splashing of water into the wax. A properly prepared wax, free from water, can be applied to a burn or granulating tissue at 150° F. without danger of pain or burning. If the wax comes in contact with water, it is more or less uncomfortable to the patient, and the wax does not adhere to the granulations as tightly as it should.

For the first two or three weeks, or until the granulated area shows a minimum of wound secretion, the dressing should be changed every twenty-four hours; because in the early stages of the wound, there is rapid sloughing and separation of the tissues. This seropurulent liquid gently lifts the wax from the wound, so that it does not adhere to the tissue and frequently the fluid escapes from the edges of the dressing. The wax shell is easily and painlessly removed by lifting it from the edges, or by making an incision with scissors and then gently peeling it off. The wound will be found to be freely bathed with lymph and purulent secretion in the early stages and this may be of offensive odor. The wound is then gently cleaned with a mild antiseptic solution. Cotton balls may be used to wipe away the secretion, or an atomizer may be used. After the wound has been thoroughly cleaned, the wax is applied as in the original dressing.

At the first dressing, burns should not be scrubbed with antiseptic solutions, nor should such solutions be used in cleaning while redressing. Saline solution,

picric acid and solutions of chlorazene may be used in cleaning. In third and fourth degree burns with great loss of tissue, granulations should be sterilized by the Carrel-Dakin method and skin grafted at the earliest possible opportunity. The advantages for the paraffin wax treatment of burns are stated as follows:

1. Relief from pain to a great degree.
2. Cleaner and more comfortable.
3. Fewer scars and contractions.
4. Skin grafting rarely necessary.
5. More rapid healing.
6. Superior in every way to methods commonly used.

—I. C. I.

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### *The Fate of the Erythrocytes*

THE length of life of the red blood cell is unknown. There is no question, however, that every erythrocyte must sooner or later undergo disintegration, a process which is thought to be ushered in by the injection of the red cell by a phagocyte in the spleen or in the hemolymph glands. The hemoglobin of the disintegrated cell is set free and carried to the liver, where it is broken up into hematin, which the body stores for future use, and into bile pigments which are excreted. This is the current explanation of the physiology of normal blood destruction. It is simple and plausible, but recent work fails to confirm the above theory.

Rous and Robertson<sup>1</sup> have examined by special methods the spleens of a variety of animals, and have failed to find evidence to confirm the belief that phagocytic destruction of the erythrocytes occurs in all animals to an extent sufficient to account for the normal destruction of blood cells. Especially is this the case in man, in the rhesus monkey, and in the cat. Seeking for extracellular means of cell destruction, these investigators have found that prolonged perfusion of the spleen and liver, and other organs to a more limited extent, with defibrinated blood or Locke's solution, causes cells to appear in the perfusate which resemble red blood cells which have lost their hemoglobin. They have found similar bodies in the blood of patients with pernicious anemia and congenital hemolytic jaundice. Nevertheless, histologic evidence together with the fact that no such blood cells occur when the perfusion is carried on for a limited time, lead them to conclude that these bodies do not represent hemolysed blood cells, but rather tissue cells. In the anemias, however, they believe that probably the bodies arise from hemolysed blood cells. From this they conclude that normally hemolysis can not play an important role in destroying the erythrocyte. They have found another and unsuspected method for blood destruction in all animals thus far studied; namely, the disintegration of the blood cells by fragmentation, while they are circulating, without loss of their hemoglobin. These fragmented cells are found most numerous in the spleen. By selective staining, the authors have been able to prove that this fragmentation is not the result of phagocytic activity. They also succeeded in increasing the number of fragmented



forms by rendering the animals plethoric by transfusions, or anemic by hemorrhage. They believe that the small ill-formed cells known as microcytes and poikilocytes, observed in severe anemias, are due to the fact that the marrow in its anemic condition is not able to produce a resistant erythrocyte, and fragmentation, therefore, takes place easily. A similar condition may exist in the severe anemias of man and account for the general high resistance in the red cells found in these patients in as much as weak cells are generally fragmented very soon after they are formed.

These authors point out that Ehrlich stated long ago that the microcytes and poikilocytes of anemia result from fragmentation of the circulating blood, but he believed that this fragmentation was a purposeful division in order to increase the total surface of the red cells. The ultimate fate of the red cell fragments is not known. It is reasonable to suppose that the broken bits containing hemoglobin are transformed into hematin and bile pigments. It is generally thought that the bile pigments serve as an index to blood destruction. However, the extensive studies of Hooper and Whipple<sup>2</sup> in the metabolism of the bile pigments have thrown new light on this question. Their experiments show that the bile pigments arise in part at least, from pigments which the liver has made in excess of its needs for the making of hemoglobin, and since it is not needed it is excreted. If this be the case, bile pigments are not a reliable index to blood destruction.

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—R. G. P.

### *Tuberculosis, Cancer, and X-Ray*

IN 1914, Lewis and Margot<sup>1</sup> published the observation that mice experimentally infected with bovine tubercle bacilli developed splenic enlargement. They also observed that animals splenectomized three weeks before infection with tubercle bacilli were more resistant than normal animals. In the same year, Murphy and Ellis<sup>2</sup> stated their belief in the conception that the lymphocyte is an important factor in resistance to tuberculous infection. In an unpublished study, Lang and Murphy,<sup>3</sup> studying the blood changes following splenectomy, showed that immediately after splenectomy the total lymphocyte count falls, but that it then commences to rise until by the nineteenth to the twenty first day, 75 per cent of the animals (50 mice) showed an average increase of 11,000 lymphocytes per c.mm.,—a gain of 100 per cent above the normal count. They thought it probable that the lymphocytosis might be a factor in causing the greater resistance displayed by the splenectomized animals. A recent series of experiments confirms this view.<sup>4</sup> These experiments showed that mice splenectomized and then exposed to repeated small doses of x ray, which affects primarily the lymphoid organs,<sup>5</sup> were more susceptible to infection than normal animals, or animals which had only been splenectomized. Also, Morton<sup>6</sup> has

observed that x-rayed guinea pigs are more susceptible to infection with the human type of tubercle bacilli than normal animals.

The recent experiments reported by Taylor and Murphy<sup>4</sup> also attack the problem in another way. Murphy and Morton<sup>7</sup> had previously shown that mice previously immunized against, and then inoculated with, a transplantable mouse carcinoma, develop a marked lymphocytic reaction which lasts several weeks. They, therefore, used such cancer immune animals and inoculated them with tuberculosis, with the result that they showed a greater resistance to bovine tuberculosis than controls. On the other hand, if in cancer immune mice the lymphocytosis is prevented or removed by x-rays, the resistance to tuberculosis is removed.

Taylor and Murphy say that they did not undertake the experiments with the idea of establishing a relationship between cancer and tuberculosis, although it has been believed that an antagonism between the two exists. Their experiments, however, suggest the basis for the popularized belief, which is probably based upon exceptional cases in which the two diseases have coexisted.

The interesting fact seems to be that in both tuberculosis and cancer the lymphoid tissue of the body may be a determining factor in resistance and yet the *modus operandi* involved may be absolutely different.<sup>8</sup>

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—P. G. W.

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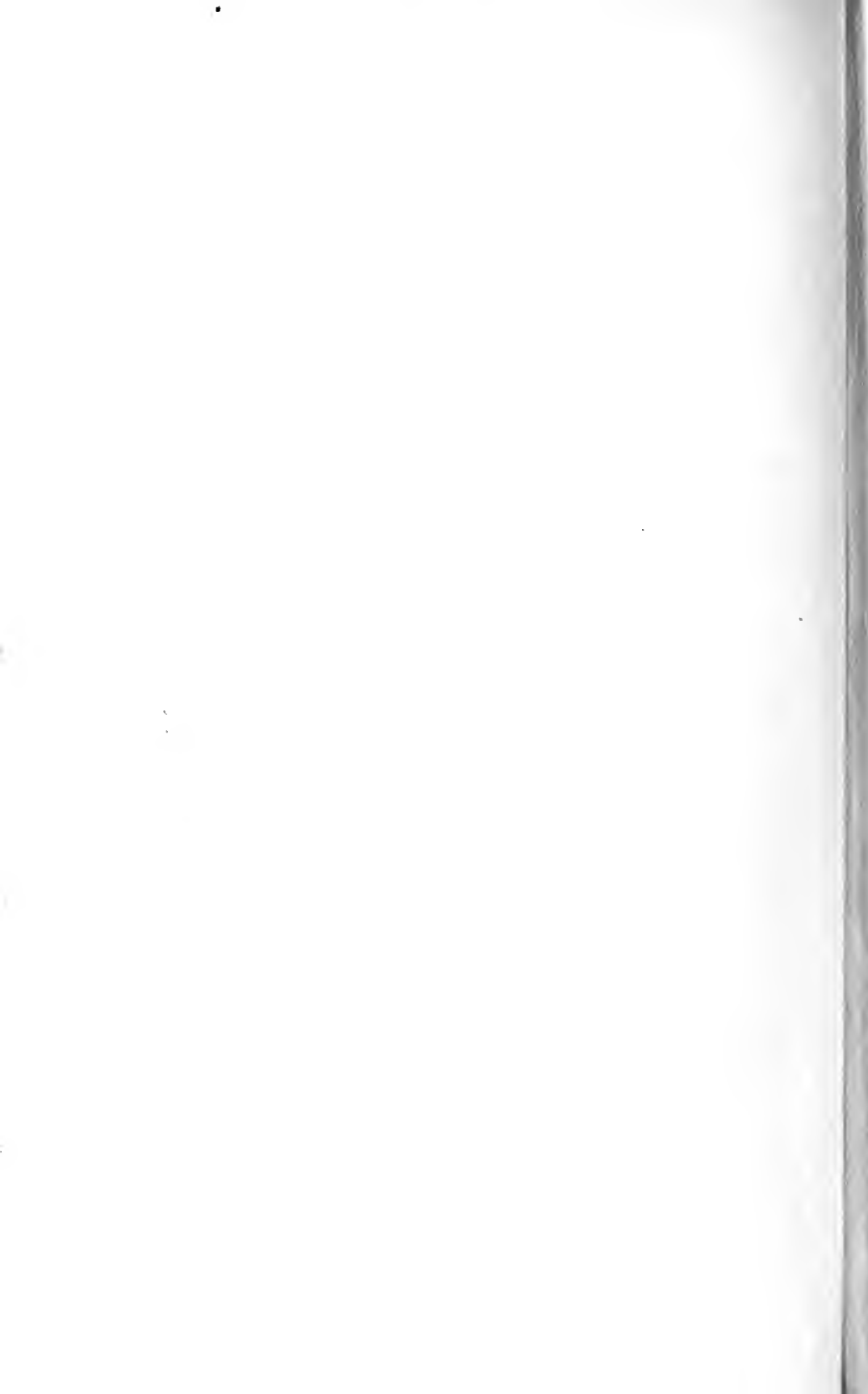
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